Glutamatergic and Dopaminergic Mechanisms of Sleep-Wake Regulation in Healthy Humans

---

Dissertation

zur

Erlangung der naturwissenschaftlichen Doktorwürde
(Dr. sc. nat.)

vorgelegt der

Mathematisch-naturwissenchaftlichen Fakultät

der

Universität Zürich

von

Sebastian Camillo Holst

aus

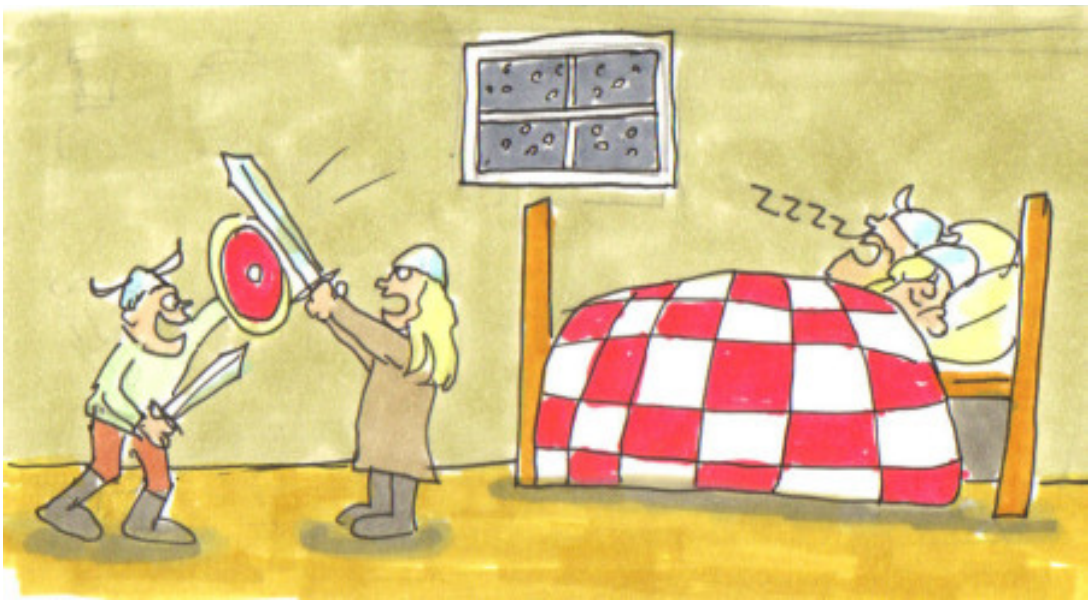
Dänemark

Promotionskomitee:
Prof. Dr. Jean-Marc Fritschy (Vorsitz)
Prof. Dr. Hans-Peter Landolt (Leitung der Dissertation)
Prof. Dr. Thierry Hennet
Prof. Dr. Peter Achermann
Prof. Dr. Mehdi Tafti

Zürich, 2013

True silence is the rest of the mind,
and is to the spirit what sleep is to the body,
nourishment and refreshment.

William Penn



“Søvn, søvn, forfærdelige opfindelse,
forfærdelige nødvendighed, du eneste magt som
jeg frygter”

Søren Kirkegaard

Preface

The scientific research reported in this thesis, was performed in the section of Chronobiology and Sleep Research, at the Institute of Pharmacology and Toxicology at the University of Zürich. This thesis would not have been possible without the support, encouragements and helpful guidance of my mentor Prof. Dr. Hans-Peter Landolt, who has provided a prosperous working environment as well as the time and space for independent thinking. To him I owe may thanks. In addition, I would like to warmly thank Prof. Dr. Peter Achermann, Prof. Dr. Alexander Borbély and Prof. Dr. Irene Tobler for sharing their knowledge and experience, and for enriching discussions in journal clubs and seminars. Your presence has been an inspiration. I thank Prof. Jean-Marc Fritschy for being my doctor father and examiner of my thesis, Prof. Dr. Thierry Hennet and Prof. Dr. Mehdi Tafti for being part of my thesis committee and for their inputs and ideas in our meetings. I also want to thank Prof. Dr Wolfgang Berger from the Institute of Medical Genetics, and Prof. Dr. Steven Brown for giving me the opportunity to work in their labs when extracting DNA and performing genetic analysis. Thanks should also go to Dr. Milan Scheidegger for his help on carrying out the PET/MRI measurement and to Dr. Roland Dürr and Karl Wütrich for technical support and help with data analysis. Moreover, I want to thank the Zürich life science graduate school, and the ZIHP in particular, for providing a framework which made it possible to carry out my PhD here in Switzerland.

Working in a human sleep lab has been an enlightenment. To gather data on human sleep, performing sleep deprivation studies, genotyping and analyzing complex data, has required the combined efforts of the entire group. For that I want to kindly thank Katharina, Valérie, Carina, Laura, Katrin, Thomas, Jurian, Leila, Alessia, Amandine, Emily, Wouter, Sandra and Michèle. Your company and friendships are of great importance to me, and something I would not have been without.

Special thoughts go to my mother, who lost her fight to cancer in Denmark, shortly after I began to work on my PhD. Without her inspiration in my life, this work would have never been possible. Thanks also go to my family and friends back home, for their continued support and encouragements. Finally I want to thank Marianna for remaining an irreplaceable source of energy and motivation.

Table of Contents

Summary

The neurochemical circuitry and molecular mechanisms underlying the regulation of sleep and waking remain an open and largely unanswered question in modern neuroscience. The ascending reticular arousal system located in the brain stem and basal ganglia, consists of a number of clearly defined cell groups with distinct neurotransmitters. These nuclei have been associated with sleep-wake regulation and predictably change their firing patterns across vigilance states. The striatum plays a central role in mediating arousal. It expresses distinct receptor heteromers, forming functional units of adenosine A_{2A} receptors, dopamine D_2 receptors, and metabotropic glutamate receptors of subtype 5 (mGluR5). The aim of the present thesis was to investigate contributions of the dopaminergic and glutamatergic neurotransmitter systems to the regulation of sleep and wakefulness. To do so, an integrative approach was applied, investigating functional genetic polymorphisms, molecular brain imaging, pharmacological interventions, polygraphic EEG recordings, actigraphy, and cognitive performance testing in healthy humans.

To investigate dopaminergic aspects of sleep-wake regulation, genotyping of two functional polymorphisms in the genes coding for the dopamine transporter (DAT) and the catechol-O-methyl-transferase (COMT) was performed in 110 healthy men and women. Both DAT and COMT regulate cerebral dopamine levels. They are primarily expressed in the striatum and prefrontal cortex, respectively. The variable-number-tandem-repeat (VNTR) polymorphism in the 3'-untranslated region of the gene encoding DAT (*DAT1*, *SLC6A3*) has been associated with 15-20 % reduced striatal expression of DAT in 10-repeat allele homozygotes (10R/10R) when compared to hetero- and homozygous 9-repeat allele (9R) carriers. On the other hand, the Val158Met polymorphism of *COMT* causes a drastic reduction in COMT enzymatic activity. Daytime sleepiness ratings and continuous motor rest-activity recordings during a roughly 4-week period revealed that male *DAT1* 9R carriers have higher overall motor activity compared to 10R/10R homozygotes, and more often report elevated sleepiness. On the other hand, *COMT* genotype did not affect rest-activity patterns, yet modulated body-mass-index (BMI) (Chapter 2).

To further investigate effects of the *DAT1* polymorphism on sleep-wake regulation, 57 subjects underwent a controlled sleep deprivation protocol, and a subgroup of participates received either caffeine (n=16) or modafinil (n=22) in a placebo-controlled, double-blind manner. Sleep deprivation-induced increases in

established neurophysiological markers of sleep need, including slow wave sleep, EEG slow-wave activity (0.5 - 4.5 Hz), and number of individual low-frequency (0.5 - 2.0 Hz) oscillations in non-rapid-eye-movement (NREM) sleep, was enhanced in 10R/10R homozygotes when compared to 9R carriers. Moreover, caffeine and modafinil affected wakefulness-induced changes in functional bands (delta, sigma and beta) of rhythmic brain activity in wakefulness and sleep in a *DAT1* genotype-dependent manner. These findings suggest that striatal dopaminergic neurotransmission is involved in sleep-wake regulation and modulates the effects of the common stimulants modafinil and caffeine (Chapter 3).

To investigate glutamatergic aspects of sleep-wake regulation, positron emission tomography (PET) imaging with the highly selective, non-competitive mGluR5 antagonist ^{11}C-ABP688 was performed in 26 healthy men. All completed two distinct imaging session, following 9 and 33 hours of sustained wakefulness. Prolonged wakefulness increased the availability of mGluR5 in human brain by roughly 3.5%. Following correction for multiple comparison ($p < 0.0038$), a significant increase after prolonged wakefulness was observed in anterior cingulate cortex, insula, medial temporal lobe, parahippocampal gyrus, striatum, and amygdala. The increase in mGluR5 availability in all these regions correlated significantly with the sleep deprivation-induced increase in subjective sleepiness. These data suggest a role for mGluR5 in homeostatic sleep–wake regulation (Chapter 4).

The mGluR5 have been linked to the generation of slow oscillations (< 1 Hz), a hallmark of the NREM sleep EEG, which has been associated with neuronal plasticity and memory processing. Pharmacological inhibition of mGluR5 has been reported to rescue Fragile X syndrome in mice, a X-chromosome linked genetic disorder in humans, caused by a CGG repeat polymorphism in the Fragile X mental retardation 1 (*FMR1*) gene underlying the complete loss of Fragile X mental retardation protein (FMRP). The loss of FMRP results in mental retardation and disturbed sleep in humans, and strongly increased sleep time in Drosophila. To examine the potential link between mGluR5 and FMRP in sleep-wake regulation, genetic analysis of the *FMR1* gene was combined with nocturnal EEG recordings and *in vivo* measurements of the availability of mGluR5 in baseline and sleep-deprived conditions. A strong association between global mGluR5 availability and EEG markers of sleep need was observed. Especially EEG slow oscillations were highly correlated with global mGluR5 availability in rested and sleep-deprived states. Furthermore, genetic analysis revealed that healthy *FMR1* odd CGG repeat number carriers have roughly

90% increased FMRP expression than even repeat number carriers. The odd number CGG repeat carriers exhibited a reduced response to sleep deprivation as observed by stagnant global mGluR5 availability, EEG slow oscillations and slow wave activity. Consequently, the data indicate that mGluR5 availability may provide a molecular marker of sleep need in humans. Additionally, mGluR5 availability may play a key role for the regulation of EEG slow oscillations in NREM sleep. Finally, the data suggest that *FMR1* genotype modulates mGluR5 availability and affects EEG makers of sleep need in healthy adult men (Chapter 5).

In summary, this thesis examined effects of dopaminergic and glutamatergic neurotransmission on sleep-wake regulation in healthy humans. It provides first evidence that dopamine modulates markers of sleep homeostasis, and that a polymorphism of *DAT1* differentially modulates distinct effects of the stimulants caffeine and modafinil. Furthermore, the work reveals that mGluR5 contribute to subjective and objective markers of sleep need. Finally, the availability of mGluR5 and EEG markers of sleep need is modulated by a distinct polymorphism of the *FMR1* gene. Overall, the findings support a significant role the DAT and mGluR5 in regulating human sleep and wakefulness.

Zusammenfassung

Die neurochemischen und molekularen Mechanismen, die der Regulation des Schlafs und des Wachzustands zugrunde liegen, sind bis heute offene und weitgehend unbeantwortete Fragen in der Neurowissenschaft. Das aufsteigende retikuläre Aktivierungssystem, welches sich im Gehirnstamm und in den Basalganglien befindet, beinhaltet eine Anzahl klar definierter Zellgruppen mit bestimmten Neurotransmittern. Diese Nuclei wurden mit der Schlaf-Wach-Regulation assoziiert und verändern ihre Feuerungsmuster in Abhängigkeit des Schlafs und Wachzustands. Das Striatum spielt eine zentrale Rolle bei der cerebralen Aktivierung. Es exprimiert verschiedene Rezeptor-Heteromere, die funktionelle Einheiten aus Adenosin A_{2A} Rezeptoren, Dopamin D_2 Rezeptoren und metabotropen Glutamatrezeptoren des Subtyps 5 (mGluR5) bilden. Das Ziel der vorliegenden Dissertation war zu untersuchen inwiefern das dopaminerge und das glutamaterge Neurotransmittersystem zur Regulation von Schlaf und Vigilanz beim Menschen beitragen. Dafür wurde eine integrative Vorgehensweise angewendet und folgende Untersuchungen kombiniert: Typisierung funktioneller, genetischer Polymorphismen; molekulare Bildgebund des Gehirns; pharmakologische Eingriffe; polygraphische EEG Aufnahmen; Aktigraphie und das Testen kognitiver Leistungen bei gesunden Versuchspersonen.

Um die dopaminergen Aspekte der Schlaf-Wachregulation zu untersuchen, wurden 110 gesunde Männer und Frauen bezüglich zweier funktioneller Polymorphismen in den Genen für den Dopamin Transporter (DAT) und die Catechol-*O*-Methyltransferase (COMT) genotypisiert. Beide, DAT und COMT regulieren gemeinsam die zerebralen Dopaminspiegel, wobei DAT primär im Striatum und COMT primär im präfrontalen Kortex zu finden sind. Der VNTR (variable-number-tandem-repeat) Polymorphismus in der 3'-untranslatierten Region des Gens, welches DAT (*DAT1, SLC6A3*) kodiert, bewirkt eine um 15-20 % verminderte DAT Verfügbarkeit im Striatum bei 10-Repeat-Allel-Homozygoten (10R/10R) im Vergleich zu hetero- und homozygoten Trägern des 9-Repeat-Allels (9R). Andererseits verursacht der Val158Met *COMT*-Polymorphismus eine drastische Abnahme der COMT Aktivität. Die Bestimmung der Tagesschläfrigkeit und die durchgehende Aufzeichnung der motorischen Aktivität während durchschnittlich 4 Wochen zeigte auf, dass *DAT1* 9R Träger eine allgemein höhere Aktivität aufweisen und öfters über eine erhöhte Schläfrigkeit berichten. Andererseits waren zwischen

den *COMT* Genotypen die Ruhe-Aktivitätsrhythmen nicht underschiedlich, dagegen hatte dieser Polymorphismus einen Einfluss auf den Body-Mass-Index (Kapitel 2).

Um die Effekte des *DAT1* Polymorphismus auf die Schlaf-Wachregulation genauer zu untersuchen, absolvierten 57 Versuchspersonen ein kontrolliertes Schlafentzugsprotokoll. Untergruppen der Teilnehmer nahmen gemäss einer placebo-kontrollierten, doppel-blinden Versuchsanordnung entweder Koffein (n=16) oder Modafinil (n=22) ein. Durch Schlafentzug induzierte Zunahmen bei etablierten neurophysiologischen Merkmalen des Schlafbedürfnisses, einschliesslich Tiefschlaf (slow wave sleep), langsam-wellige Hirnstromaktivität (0.5-4.5 Hz) und der Anzahl individueller niedrig-frequenter (0.5-2.0 Hz) Oszillationen im non-rapid-eye-movement Schlaf (NREM) waren bei den *DAT1* 10R/10R im Vergleich zu den 9R Trägern erhöht. Des Weiteren führten Koffein und Modafinil durch die verlängerte Wachzeit zu induzierten Veränderungen in funktionalen Frequenzbändern (delta, sigma, beta) der rhythmischen Hirnaktivität im Wachzustand und im Schlaf gemäss dem *DAT1* Genotyp. Diese Ergebnisse deuten darauf hin, dass sich Einflüsse der striatalen, dopaminergen Neurotransmission auf die Schlaf-Wachregulation und auf die Effekte der weit verbreiteten Stimulanzien Modafinil und Koffein auswirken (Kapitel 3).

Um glutamaterge Aspekte der Schlaf-Wach-Regulation zu untersuchen, wurde bei 26 gesunden Männern die Methode der Positron Emissionstomographie (PET) mit dem hoch selektiven, nicht-kompetitiven mGluR5 Antagonisten ^{11}C-ABP688 angewendet. Alle Probanden nahmen an zwei individuellen Bildgebungssitzungen teil, einmal nach 9 und einmal nach 33 Stunden anhaltender Wachzeit. Die Verlängerung der Wachzeit bewirkte eine Erhöhung der mGluR5 Verfügbarkeit im Gehirn um circa 3.5%. Nach Bonferroni-Korrektur ($p < 0.0038$) zeigte sich nach anhaltendem Schlafentzug eine signifikante Zunahme im anterioren Cingulum, in der Inselrinde, im medialen Teil des vorderen Temporallappens, im Gyrus parahippo-campalis, im Striatum und im Mandelkern (Amygdala). Die Zunahme in der mGluR5 Verfügbarkeit in all diesen Regionen korrelierte signifikant mit der schlafentzugs-induzierten Zunahme der subjektiven Schläfrigkeit. Diese Daten zeigen eine Rolle der mGluR5 beim homöostatischen Aspekt der Schlaf-Wachregulation (Kapitel 4).

Die mGluR5 werden mit der Generierung von langsamen Hirnwellen (< 1 Hz) in Verbindung gebracht, ein typisches Merkmal des NREM-Schlaf EEGs, welches mit neuronaler Plastizität und der Verarbeitung von Erinnerungen assoziiert wird. Durch

pharmakologische Blockierung der mGluR5 konnte das Fragiles-X-Sydrom (FXS) in Mäusen aufgehoben werden. Das FXS ist eine X-chromosomal assoziierte humane Erkrankung, die durch eine Mutation hervorgerufen wird, die zur Verfielfältigung eines CGG-Motivs im *FMR1*-Gen (*fragile-X mental retardation 1 gene*) und den kompletten Verlust des FMRP (fragile-X mental retardation protein) führt. Der Verlust des FMRP resultiert in mentaler Retardierung und gestörtem Schlafverhalten im Menschen und stark erhöhter Schlafdauer bei der Fruchtfliege Drosophila. Um die mögliche Verbindung zwischen mGluR5 und FMRP bezüglich Schlaf-Wach-Regulation zu untersuchen, wurden genetische Analysen des FMR1-Gens mit nächtlichen EEG Aufnahmen und *in vivo* Messungen der mGluR5 Verfügbarkeit nach normalem Schlaf und nach Schlafentzug kombiniert. Es wurde ein starker Zusammenhang zwischen globaler mGluR5 Verfügbarkeit und EEG Merkmalen des Schlafbedürfnisses beobachtet. Insbesondere langsame Hirnwellen im EEG korrelieren mit der globalen mGluR5 Verfügbarkeit sowohl im ausgeruhten als auch im schlafentzogenen Zustand. Darüber hinaus haben genetische Analysen ergeben, dass gesunde FMR1 Träger mit ungerader Anzahl an CGG-Wiederholungen eine 90 % erhöhte FMRP Expression aufweisen als Träger mit gerader Anzahl an CGG-Wiederholungen. Die Träger mit ungerader Anzahl an CGG-Wiederholungen zeigten eine verminderte Antwort auf Schlafentzug, insbesondere bei der globalen mGluR5 Verfügbarkeit, den langsamen Hirnwellen und dem Tiefschlaf. Folglich zeigen diese Daten, dass die mGluR5 Verfügbarkeit möglicherweise einen molekularen Marker für das Schlafbedürfnis darstellt. Zusätzlich spielt die mGluR5 Verfügbarkeit eine Rolle für die Regulierung von langsamen Hirnwellen im EEG während NREM-Schlaf. Schliesslich weisen die Daten darauf hin, dass der FMR1-Genotyp die mGluR5 Verfügbarkeit moduliert und EEG Marker für Schlafbedürfnis in gesunden, erwachsenen Männern beeinflusst (Kapitel 5).

Diese Dissertation untersucht den Einfluss dopaminerger und glutamaterger Neurotransmission bei der Schlaf-Wach-Regulation in gesunden Menschen. Sie liefert Hinweise darauf, dass Dopamin die Schlafhomöostase moduliert und dass ein Polymorphismus im *DAT1*-Gen den Effekt der Stimulanzien Koffein und Modafinil auf unterschiedliche Weise beeinflusst. Zusätzlich zeigt die Studie, dass mGluR5 zu subjektiven und objektiven Merkmalen des Schlafbedürfnisses beitragen. Die Verfügbarkeit der mGluR5 und EEG Marker des Schlafbedürfnisses sind durch einen bestimmten Polymorphismus im FMR1-Gen moduliert. Insgesamt weisen die Ergebnisse dieser Dissertation auf eine signifikante Rolle von DAT und mGluR5 auf die Schlaf-Wachregulation im Menschen hin.

Abbreviations

τ_d	Time constants
5HT	Serotonin / 5-hydroxytryptamine
9R	9-repeat allele
10R	10-repeat allele
10R/10R	10-repeat allele homozygotes
^{11}C-ABP688	3-(6-Methyl-pyridin-2-ylethynyl)-cyclohex-2-enone-O-11C-methyl-oxime
A_1	Adenosine A_1 receptor
A_{2A}	Adenosine A_{2A} receptor
ACh	Acetylcholine
ADA	Adenosine deaminase
ADHD	Attention deficit/hyperactive disorder
ANOVA	Mixed-model analyses of variance
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ARAS	Ascending reticular arousal system
BDNF	Brain-derived neurotropic factor
BF	Basal forebrain
BG	Basal ganglia
BMI	Body mass index
bp	Base pair
Ca	Calcium
CNS	Central nervous system
COMT	Catechol-*O*-methyltransferase
C_t	Average radioactivity concentration
D_1	Dopamine D_1 receptor
D_2	Dopamine D_2 receptor
DA	Dopamine
DAG	Diacyl glycerol
DAT	Dopamine transporter
DNA	Deoxyribonucleic acid
DV	Distribution volume
DV_{norm}	Normalized distribution volume
DZ	Dizygotic
EAAT	Excitatory amino acid reuptake transporters
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
EEG	Electroencephalogram
EOG	Electrooculogram

EMG	Electromyogram
EPSP	Excitatory postsynaptic potentials
ESS	Epworth sleepiness scale
FFT	Fast-Fourier transformation
FMR1	Fragile X mental retardation 1 gene
FMRP	Fragile X mental retardation protein
FXTAS	Fragile X tremor ataxia syndrome
GABA	Gamma-Aminobutyric acid
G-protein	guanosine nucleotide-binding protein
GP	Globus palidus
GWA	Genome wide association study
His	Histamine
Homer1a	Homer protein homolog 1a
HVA	Homovanilic acid
IP3	Inositol triphosphate
IPSP	Inhibitory postsynaptic potentials
IS	Inter-daily stability
IV	Intra-daily variability
K	Potassium
KSS	Karolinska sleepiness scale
L5	Least active 5-hours
LC	Locus coeruleus
LH	Lateral hypothalamus
LDT	Laterodorsal tegmental nuclei
LTD	Long-term depression
LTP	Long-term potentiation
M10	10-most active hours
MAO	Monoamine oxidase
MB-COMT	Membrane-bound COMT
MCH	Melanin concentrating hormone
MCTQ	Munich chronotype questionnaire
MDD	Major depressive disorder
mGluR	Metabotropic glutamate receptors
MNI	Montreal Neurological Institute brain atlas
mPFC	Medial prefrontal cortex
MR	Magnetic resonance
MRI	Magnetic resonance imaging
MWT-B	Mehrfachwahl wortschatz intelligenz Test - B
MZ	Monozygotic
Na	Sodium

NAc	Nucleus accumbens
NCBI	National Center for Biotechnology Information gene numbers
NE	Norepinephrine
NET	Norepinephrine transporter
NMDAR	and N-methyl-d-aspartate receptors
NPCRA	Non-parametric circadian rhythm analysis
NREM	Non-rapid eye movement
ORX	Orexin (hypocretin)
PB	Parabrachial nucleus
PCR	Polymerase chain reaction
PD	Parkinson's disease
PER3	Period circadian protein homolog 3
PET	Positron emission tomography
PMOD	Pixel-wise image modeling
POMS	Profile of Mood States
PPT	Pedunculopontine tegmental nuclei
Process C	Circadian process of the two-process model
Process S	Homeostatic process of the two-process model
PSG	Polysmonography
PVT	Psychomotor vigilance task
RA	Relative amplitude
REM	Rapid eye movement
RNA	Ribonucleic acid
RT	Reaction time
sc	Sleep control
S-COMT	Soluble COMT
SCN	Suprachiasmatic nucleus
sd	Sleep deprivation
SEM	Standard error of the mean
SHY	Synaptic homeostasis hypothesis
SN	Substantia nigra
SNP	Single nucleotide polymorphism
STAI	State-trait anxiety inventory
STD	Standard deviation
SUV	Standard uptake value
SWA	Slow wave activity
SWS	Slow wave sleep
TAC	Tissue time activity curve
TH	L-tyrosine hydroxylase
TIB	Time spent in bed

TMN	Hypothalamic tuberomammillary nucleus
TSS	Tiredness symptoms scale
UTR	Untranslated region
VGluT	Vesicular glutamate transporter
VLPO	Ventrolateral preoptic nucleus
V_{ND}	Nondisplaceable volume of distribution
VNTR	Variable number tandem repeat
VOI	Volume of interest
VP	Ventral palidum
vPAC	Ventral periaqueductal gray
V_t	Total distribution volume
VTA	Ventral tegmental area
WASO	Wakefulness after sleep onset

Chapter 1

Introduction

Sleep and the electroencephalogram

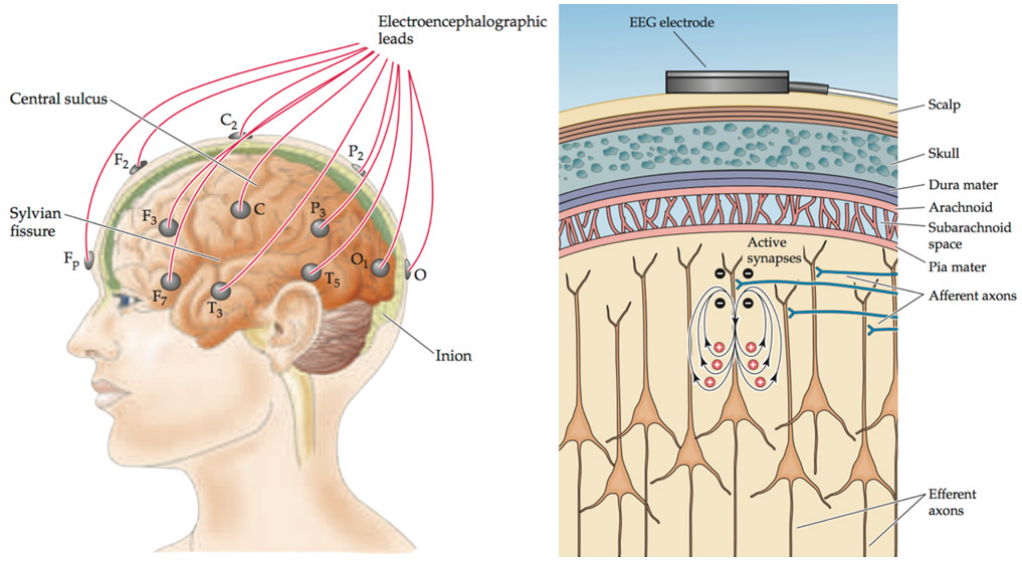
Sleep is a highly regulated and global state controlled by the brain. In its basic form, it is characterised by a reduced awareness to external stimuli, combined with an increased sleep intensity following deprivation. By this definition, sleep is observed throughout the animal kingdom and appears to have been conserved throughout evolution (Campbell and Tobler, 1984; Tobler, 2011). In the past, sleep was believed to be a passive state associated with memory impairments and loss of consciousness. However, following the discoveries of rapid eye movement (REM) sleep (Aserinsky and Kleitman, 1953) and later, the cyclic alternations between REM and non-REM (NREM) sleep (Dement and Kleitman, 1957), it was generally accepted that sleep is an active process.

Self-reported sleep duration among more than 1.1 million American adults revealed a median daily sleep duration of 8 hours, which accounts for approximately 1/3 of our daily life (Kripke et al., 2002). Nevertheless, the importance and molecular mechanism(s) of sleep still remain poorly understood (Cirelli and Tononi, 2008). Indeed, as pioneering sleep researcher Prof. Allen Rechtschaffen commented: “If sleep doesn't serve an absolutely vital function, then it's the biggest mistake the evolutionary process ever made”.

Modern sleep research is to a large extent based on the development of the human electroencephalogram (EEG), which was first used by the German psychiatrist Hans Berger in the 1920s (Berger, 1929). Building on Berger’s pioneering work, the EEG today is an important tool for non-invasive investigation of the human brain. Standardized methods such as the 10-20 system ensures inter-individual comparability for placing EEG electrodes, with a between electrode distance of 10% to 20% (Figure 1 left). The EEG is generated by rapid depolarization of neuronal membranes, mediated by sodium and potassium voltage-dependent ion conductance, and by neurotransmitter-dependent synaptic activation. As a result, action potentials propagate along axons and dendrites. Since there is no local

accumulation of charge, current flow in one direction, is compensated by opposite current flows elsewhere. As a result, excitatory or inhibitory postsynaptic potentials (EPSP and IPSP respectively) are generated. These alternating electric fields can be detected on the scalp surface (Figure 1 right) and are known as field potentials, which represent summated activity from a large number of neurons and can be recorded with at great temporal resolution. Field potentials measured by the EEG are primarily generated by synaptic activity in apical dendrites of cortical pyramidal neurons, oriented perpendicular to the cell surface (Westbrook, 2000).

Figure 1: EEG generation and the typical 10-20 montage.



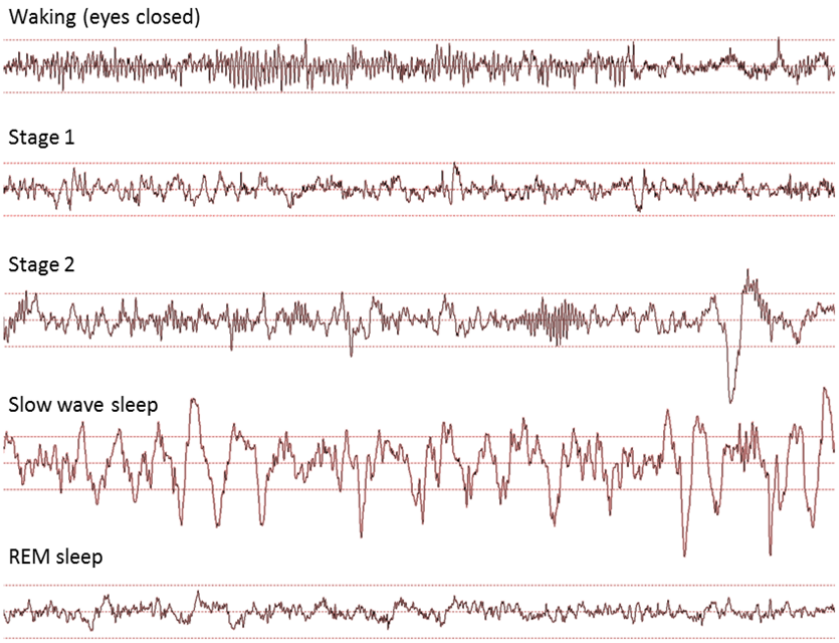
Left: Example of an EEG montage using the 10-20 system. Electrodes are positioned according to well-defined criteria with an electrode distance of 10% to 20% of the total frontal-occipital and left-right distance of the skull. Potential differences between electrodes are measured and EEG recordings therefore require at least two electrodes. Right: The EEG measures field potentials, the combined electrical activity of a large number of cortical pyramidal cells. The activity originates primarily from the apical dendrites orientated perpendicular to the cell surface. Figure from Purves, 2004.

Quantification of the EEG during wakefulness and sleep

The human EEG, together with recordings of the electrooculogram (EOG), electromyogram (EMG) and electrocardiogram (ECG) can discriminate between the vigilance states of REM sleep, NREM sleep, and wakefulness. The EEG is typically divided into bands of waves including slow oscillations (< 1 Hz), delta (1 - 4 Hz), theta (4 – 7.5 Hz), alpha (8 – 13 Hz) and beta (14 - 30 Hz) waves, although these definitions vary considerably across literature. Slow oscillations are commonly used for the sleep EEG only. Similarly, beta activity during sleep is typically defined at a slightly higher frequency (15-30 Hz) to encompass spindles/sigma (12-15 Hz)

activity. Vigilance states, as illustrated on Figure 2, can systematically be defined according to the criteria developed by Rechtschaffen and Kales in the 1960s (Rechtschaffen and Kales, 1968). According to their criteria, rested wakefulness (with eyes closed) is typically associated with distinct low amplitude alpha activity. NREM sleep is divided into stages 1 – 4 of increasing intensity. Specifically, stage 1 sleep is considered a transitional state between wakefulness and sleep. It is short-lived and characterized by irregular low voltage, mixed frequency EEG activity, associated with a dissipation of alpha waves. Stage 2 sleep is recognized by the transient occurrence of K-complexes and 11 - 15 Hz waxing and waning sleep spindles. Stages 3 and 4 contain moderate (20-50%) and high (>50%) amounts of slow waves respectively, with frequencies in the delta range and a peak-to-peak amplitude of >75 μV. Stage 3 and 4 are often combined into so-called slow wave sleep (SWS). Finally REM sleep, also known as paradoxical sleep, can be recognized by its mixed-frequency EEG activity that resembles active wakefulness. Nevertheless, REM sleep is associated with strong atonia, where only the eye muscles reveal rapid activity.

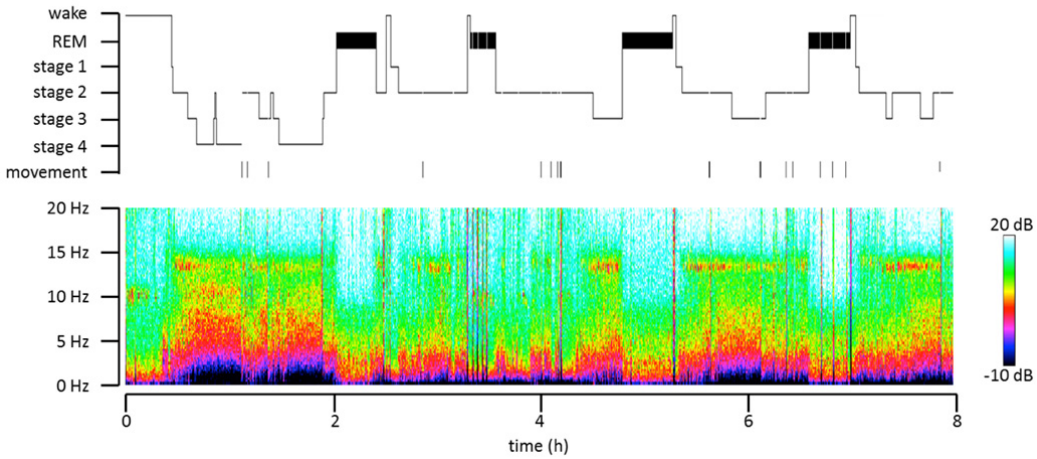
Figure 2: Example of typical EEG oscillations during wakefulness and sleep



EEG traces (20 s) recorded during a typical 8-hour sleep episode in a representative individual. Waking EEG with eyes closed can be recognized by strong alpha activity around 10 Hz. Stage 1 sleep show irregular low voltage, mixed frequency EEG. Stage 2 sleep is recognized by the common spindles (~13.5 Hz) and large K-complexes. Slow wave sleep (stage 3 and 4) is illustrated by large slow waves (>75 μV) in the delta frequency range. REM sleep is associated with mixed frequency, low amplitude waves. Furthermore, REM sleep is characterized by reduced muscle tone and rapid eye movements recognized in the EMG and EOG (not illustrated here). Dotted lines illustrates the amplitude criteria for slow waves; ± 37.5 μV.

Despite the elegance of the sleep stage criteria defined by Rechtschaffen and Kales, scoring is based on visual inspection of the EEG in 20 – 30 s epochs, and can vary considerably even among experienced sleep scorers (Norman et al., 2000). A more objective way to quantify EEG activity is to apply fast-Fourier transformed (FFT) spectral analysis. This mathematical transformation converts the EEG signal from time to frequency domain (Cooley and Tukey, 1965). The FFT requires a stationary signal and is therefore performed in time windows of either 2 or 4 s for wakefulness and sleep, respectively. This allows for frequency resolutions of 0.25 Hz for sleep recordings and 0.5 Hz for wakefulness with a spectrum depicting spectral power (μV^2/Hz) for a specified time frame (e.g. across NREM sleep) (Figure 3).

Figure 3: Illustration of an 8-hour sleep EEG recording



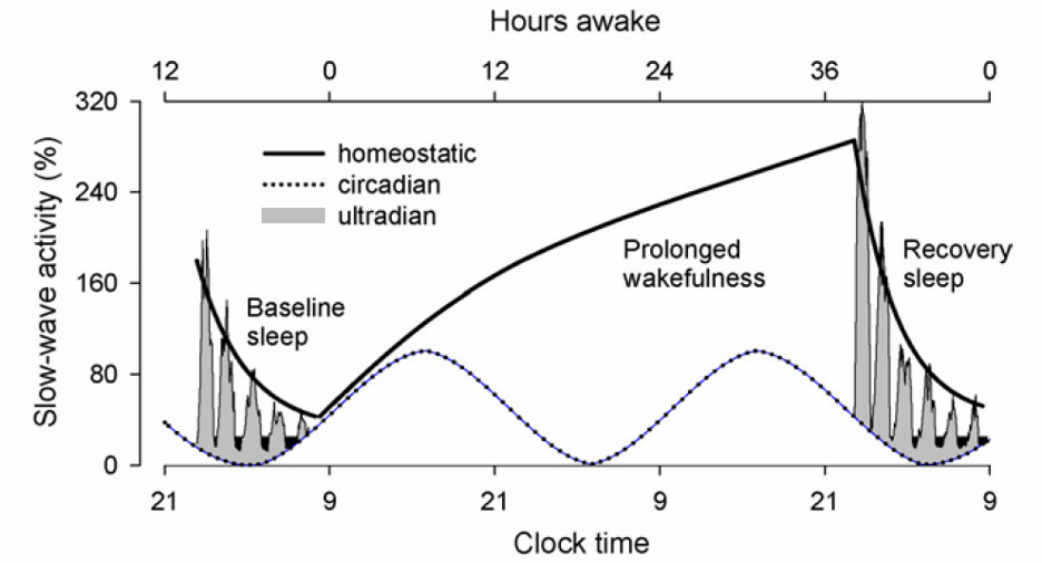
Sleep recorded during a typical 8-hour sleep episode in a representative individual. At least four NREM-REM cycles can be identified, lasting 1.5 to 2 h, typically followed by short awakenings. Upper panel: A hypnogram illustrating 20 s of visually scored sleep epochs. Lower panel: Spectral power (0 - 20 Hz) colour coded on a logarithmic scale. Colour coding: 0 dB = 1 μV^2/ Hz.

Conceptual framework of the regulation of sleep and waking

Sleep homeostasis is a founding principle of sleep-wake regulation. It was conceptualized in the 1980s by Alexander A. Borbély, when he first proposed the two-process model of sleep regulation, a concept widely accepted today (Borbély, 1982; Achermann and Borbély, 2011). The two-process model describes the interaction between the homeostatic process S and the circadian process C. Together they generate the timing of sleep and wakefulness. Process S mediates the rise in sleep propensity, or sleep pressure, during wakefulness and the decline during sleep. If sleep is reduced or absent, sleep propensity is augmented, whereas excess sleep is followed by a reduction in sleep propensity. Based on human sleep

EEG recordings, process S has been linked to slow wave activity (SWA) in the delta range (specifically between 0.5-4.5 Hz). Pooled data across individuals revealed that SWA decline during nocturnal sleep in a way that can be described mathematically by an exponential function (Daan et al., 1984; Achermann et al., 1993; Achermann and Borbély, 2011) (Figure 4). Recently, the decline in SWA was also successfully simulated in single individuals (Rusterholz et al., 2010). During wakefulness, process S is modelled as a saturating exponential increase.

Figure 4: The two-process model of sleep-regulation



The two-process model of sleep regulation, conceptualized by A.A. Borbély in 1982. Schematic view of the interaction between the homeostatic process S and the circadian process C, during a normal night of sleep and following 40 hours of prolonged wakefulness. The interaction between process S and C is proposed to generate the timing of sleep and waking. The ultradian process depicted illustrates EEG SWA as it declines across consecutive NREM sleep episode. Figure from Landolt, 2008.

The circadian process C is regulated by the suprachiasmatic nucleus (SCN) in the hypothalamus, which consists of roughly 20.000 neurons (Moore and Eichler, 1972; Stephan and Zucker, 1972). The SCN functions as an endogenous zeitgeber and its outputs can be described by a sine-wave function with a period of roughly 24 hours, operating virtually independent of prior sleep or wakefulness. The SCN is mainly entrained by light, detected by retinal ganglion cells and transmitted via melanopsin-releasing neurons of the retinohypothalamic tract (Gooley et al., 2001; Berson et al., 2002). However, other zeitgebers such as temperature (Blake, 1967), and feeding behaviour (Richter, 1922), can also modulate the endogenous clock. The actual timekeeping is maintained by interconnected SCN neurons and clock gene transcription feedback loops (Albrecht and Eichele, 2003).

The interaction between process S and process C regulates timing and duration of sleep, but also alertness and maintenance of wakefulness in the waking phase (Van Dongen and Dinges, 2003). Sleep deprivation is a demanding yet useful tool that allows for experimental disentanglement of the two processes, even in human subjects. Following a period of circadian entrainment to a specific bedtime, sleep at "baseline" levels can be compared with recovery sleep following 40 hours of prolonged wakefulness. This allows for comparison of sleep at different homeostatic levels, yet at the same circadian time point, thereby effectively eliminating the effects of process C. When the response to sleep deprivation is calculated intra-individually, it effectively reduces inter-individual differences in sleep intensity and architecture, which allows for more accurate between-subject comparisons. Furthermore, sleep is a highly controlled state characterized by the loss of consciousness. This again provides researchers with a long and "undisturbed" time window to examine the brain. As highlighted in the next section on genetics, this may reveal why the sleep EEG is one of the most hereditary features known in humans and can explain why sleep is an excellent tool for studying genetic influences.

Genetic influences impacting sleep-wake regulation

Genetic studies investigating sleep and the sleep EEG can be traced back as far as the 1930s where Geyer investigated sleep, by comparing "sleep habits" among monozygotic (MZ) and dizygotic (DZ) twins. In his paper, Geyer reported higher concordance among MZ than among DZ twins (Geyer, 1937). More than half a century later, modern day analysis of the sleep EEG corroborates the initial findings of Geyer. Comparisons of the NREM sleep EEG between MZ and DZ twins revealed, that theta and alpha frequencies, but also to a lesser extent delta waves and spindles, are genetically modulated (Ambrosius et al., 2008). In a large study cohort of 80 twin pairs, de Gennaro and colleagues reported a heritability estimate of up to 96% for 8 – 16 Hz EEG activity in NREM sleep (de Gennaro et al., 2008), a finding also supported by earlier studies (Buckelmüller et al., 2006). Not only the sleep EEG shows high heritability estimates. Also alpha activity during waking, investigated among 213 twin pairs, was reported to have a heritability estimate of up 89% (Van Beijsterveldt et al., 1996). Overall, this supports the trait-like nature of human sleep and the EEG.

Although genes have been conclusively established to play a role in regulating sleep and the EEG, the search for the specific genes involved has only recently been

the subject of investigation. One successful approach to shed light on this question, has been the so-called candidate gene approach, where a specific gene or single nucleotide polymorphism (SNP) is examined based on *a priori* knowledge of the functional effect, role and importance of that specific genetic factor. Hence, the candidate gene approach has high statistical power, but requires strong and valid hypotheses to be powerful. Otherwise, "noise" from other genetic factors may eliminate potential findings, or even lead to false discoveries (Zhu and Zhao, 2007; Wilkening et al., 2009; Amos et al., 2011). Within the sleep field, the candidate gene approach has so far been successfully used to establish the role of a handful of polymorphisms. One of which is the functional role of 3' untranslated region (UTR) variable number tandem repeat (VNTR) polymorphism of the *PER3* gene coding for the period circadian protein homolog 3. *PER3* has so far been associated with diurnal preference, delayed sleep phase syndrome, differences in SWA, cognitive performance and modulations of body mass index (BMI) (Archer et al., 2003; Viola et al., 2007; Lázár et al., 2012). Similarly, SNPs in the genes coding for adenosine deaminase (*ADA*) and brain-derived neurotropic factor (BDNF) have been shown to affect markers of sleep homeostasis (Rétey et al., 2005; Bachmann et al., 2012a; 2012b). Moreover, a polymorphism in the *ADORA2A* gene coding for the adenosine A_{2A} receptor has been shown to modulate individual sensitivity to caffeine (Rétey et al., 2005; 2007); whereas the functional Val158Met polymorphism of the Catechol-*O*-methyltransferase *(COMT)* gene (SNP-ID: rs4680), has been reported to modulate the effects of the stimulant modafinil (Bodenmann et al., 2009b; Bodenmann and Landolt, 2010).

Compared to the candidate gene approach, genome wide association studies (GWAs) use DNA chips to establish the effects of a large number of SNPs simultaneously. GWAs do not require *a priori* knowledge and are capable of discovering new genetic associations. However it suffers from low statistical power since it examines millions of SNPs, resulting in an alpha level of around 5×10^{-8} (Amos et al., 2011). Therefore, unless the study sample is very large, GWAs may be best suited for detecting SNPs that pose greater effects. (Manolio et al., 2009; Amos et al., 2011). In that respect, the candidate gene approach remains a useful tool, along side GWAs, for disproving or confirming specific hypotheses.

Current hypotheses for the "function(s) of sleep"

As previously mentioned, the functions and molecular mechanisms underlying

sleep-wake regulation are not yet fully understood. Although these functions remain a topic of debate and are beyond the scope of this thesis to systematically cover, the theories include: the detoxification of the brain from free radicals, glycogen replacement, immune system repair, as well as memory consolidation and the maintenance of synaptic homeostasis (Tononi and Cirelli, 2006; Rasch and Born, 2013). Given that sleep is conserved across species and associated with reduced responsiveness to the surrounding environment, sleep is generally assumed to be of the brain, by the brain and for the brain (Hobson, 2005).

The synaptic homeostasis hypothesis (SHY), proposed by Tononi and Cirelli, can be considered an elaboration of the two-process model of sleep regulation. The hypothesis proposes that wakefulness is accompanied by long-term potentiation (LTP) in a large fraction of cortical circuits, and that sleep is necessary to renormalize the net synaptic weight (Tononi and Cirelli, 2003; 2006). The enhanced synaptic weight associated with wakefulness, is then directly linked to the amount of SWA (and generalized synaptic connectivity) observed in subsequent sleep. As a consequence, sleep is a state of downscaling, enhancing the signal-to-noise ratio of encoded information. Substantial evidence for this theory now exists. For instance, molecular markers of synaptic plasticity such as BDNF and Homer1a in rats, increase during waking and decline during sleep. Furthermore, the availability of BDNF following sleep deprivation, was associated with the rebound in SWA (Huber et al., 2007). Moreover, areas that have been “used” extensively during waking show higher SWA in subsequent sleep (Huber et al., 2004). Similarly, synapse number and size was increased during wakefulness and reduced during sleep in Drosophila kept in an enriched environment (Bushey et al., 2011). Finally, cortical metabolic rates in mice increase as a consequence of sleep deprivation and decrease with sleep (Vyazovskiy et al., 2008b). However, a major criticism of the SHY is how sleep is stringently assumed to be associated with downscaling - a state where LTP should not occur (Frank, 2012; Tononi and Cirelli, 2012). Indeed, some evidence rather suggest enhanced synaptic transmission during sleep (Ramm and Smith, 1990; Nakanishi et al., 1997; Cirelli et al., 2004). Furthermore, the upscaling or “learning”, assumed to happen during waking, may not solely rely on LTP. Instead, “learning” has been associated equally with long-term depression (LTD) and LTP-type synaptic plasticity (Kemp and Manahan-Vaughan, 2007; Frank, 2012).

The sleep and memory consolidation hypothesis assumes that sleep enhances the formation of memories by an active and highly selective process (Diekelmann

and Born, 2010; Rasch and Born, 2013). Memory consolidation is suggested to happen from a repeated reactivation of new memories during SWS, which leads to the strengthening and transfer of memory traces, from short-term storage in the hippocampus, to long-term storage in the cortex. Because memory consolidation is assumed to be an active process, unlike the SHY, it is not essential whether sleep is associated with LTP- or LTD-types of synaptic plasticity. Rather, a memory improvement is expected as a consequence of sleep. Methodologically however, this is hard to prove since time is almost always associated with memory deterioration, independent of sleep or wakefulness. Nevertheless, it has been elegantly shown that memory traces, and the hippocampal bold-response, can be enhanced during sleep using odor-cues, resulting in “less forgetting” (Rasch et al., 2007; Diekelmann et al., 2011). Furthermore, the induction of slow oscillating potential fields to the human scalp, enhance memory recall of word-pairs following a night of sleep (Marshall et al., 2006). Finally, the duration of SWS in a post learning nap session, could predict how well pictures were remembered in a recall session 3 months later (Takashima et al., 2006). Combined, these data illustrate that sleep and SWS may be associated with memory consolidation. Nevertheless, the concept of “reduced forgetting” observed in these studies is somewhat problematic, since it makes it difficult to distinguish the specific effects of sleep from the effects of passing time.

To summarize, several hypotheses have been suggested which may partially explain the need for sleep, although they all still lack substantial evidence and are not yet generally accepted. Furthermore, it should be stressed that sleep is most unlikely to serve a single function, but rather, would be expected to play an important role for a number of homeostatic processes

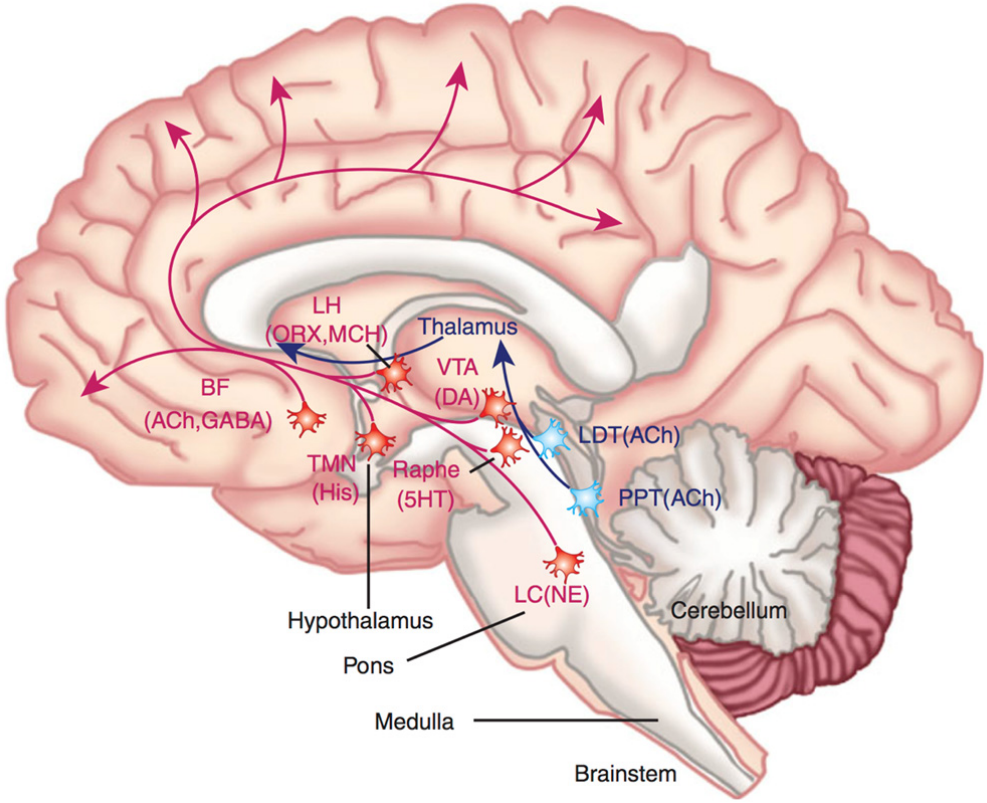
Neuronal pathways involved in sleep regulation

The principle of ‘sleep homeostasis’ is one of the most important principles of sleep regulation. Nevertheless, the biochemical and molecular mechanisms underlying sleep homeostasis are only poorly understood. However, scientific studies have begun to unravel some of the neuronal pathways that contribute to the regulation of vigilant states. These major neuronal systems influencing sleep and waking are congregated in the so-called ascending reticular activation system (ARAS) in which several distinct neurotransmitter dependent cell groups play a key role. These include nuclei of the brain stem, but also nuclei from the striatum, ventral thalamus, substantia nigra (SN), globus palidus (GP) and basal forebrain, commonly

denoted as the basal ganglia (BG). The activating role of the BG was initially advocated by the work of von Economo (1876 - 1931), who studied patients with viral *Encephalitis lethargica* (Economo, 1930). The infection resulted in lesions to the midbrain and limbic system. Specifically, von Economo could show that lesions to the midbrain ventral periaqueductal gray (vPAC) and posterior hypothalamus were followed by hypersomnolent symptoms. On the other hand, lesions to the anterior hypothalamus that extend into the BG were associated with symptoms of insomnia. The activating pathways of the central nervous system (CNS) was further investigated by Bremer and Magoun, whose work let to the first conceptual evidence for the ARAS (Bremer, 1935; Moruzzi and Magoun, 1949). Today, the ARAS incorporates arousal pathways originating from cell groups of the lateral hypothalamus (LH), BG and upper brain stem, thus including several distinct neurotransmitter systems (Saper et al., 2001; Hobson and Pace-Schott, 2002; Saper et al., 2005; Fort et al., 2009; Fuller et al., 2011; Edlow et al., 2012) (Figure 5).

Two major pathways are traditionally assigned to the ARAS. The thalamocortical projections (blue pathway, Figure 5) consist of neurons releasing acetylcholine (ACh) that project from the pedunculopontine (PPT) and laterodorsal tegmental nuclei (LDT) to the thalamus, thereby activating thalamic relay neurons and reticular thalamic cells, crucial for transmission of information via the thalamus to the cortex. The second pathway has prevailing cortical projections (red pathway, Figure 5) originating from the widely projecting neurotransmitter cell groups. They include norepinephrine (NE) releasing neurons from the locus coeruleus (LC), serotonin (5HT) releasing neurons from the raphe nucleus, dopamine (DA) releasing neurons from the ventral tegmental area (VTA), histamine (His) releasing neurons from the hypothalamic tuberomammillary nucleus (TMN), ACh releasing neurons from the basal forebrain (BF), as well as orexin (ORX) and melanin concentrating hormone (MCH) releasing neurons from the LH. All these projections show complex modulatory functions, but all promote waking behavior (Saper et al., 2005; Schwartz and Roth, 2008; Edlow et al., 2012). Nevertheless, the cortical pathway (red) also holds gamma-Aminobutyric acid (GABA) releasing neurons, which has strong projections from the ventrolateral preoptic nucleus (VLPO) inhibiting the ARAS and promoting sleep (Saper et al., 2005; Schwartz and Roth, 2008; Edlow et al., 2012).

Figure 5: The major neurotransmitter pathways of the ascending reticular arousal system (ARAS).



The ascending arousal system (ARAS) has two main pathways for arousal projecting via the thalamus (dark blue) and directly to the cortex (red). Inhibitory projections from GABA'ergic neurons of the ventrolateral preoptic nucleus (VLPO) inhibit the ARAS and promote sleep. From Nestler et al., 2009a.

The ARAS neurotransmitter pathways demonstrate firing patterns that depend on vigilance states. Strong neuronal firing during waking, decreased firing during NREM sleep, and almost silent neuronal activity during REM sleep, is observed in the raphe nucleus (Portas et al., 1998), LC (Takahashi et al., 2010) and for ORX and TMN neurons from the LH (Lee et al., 2005; Takahashi et al., 2006). ACh releasing neurons of the PPT and LDT are active during waking and REM sleep, whereas they decrease firing during NREM sleep (Steriade et al., 1990a). The VLPO cluster, on the other hand, is mainly active during sleep allowing it to inhibit the ARAS (Sherin et al., 1996; Saper et al., 2005).

Although the ARAS provides a practical framework for the established pathways modulating sleep and waking, functional brain imaging studies, applying on different methodologies, have consistently suggested additional regions such as the ventro-medial prefrontal cortex, the insula, anterior cingulate cortex and precuneus to be important for the regulation of slow waves and NREM sleep (Maquet et al., 1997;

Dang-Vu et al., 2008; Murphy et al., 2009). These brain structures appear to actively contribute to the physiological processes underlying sleep homeostasis (Dang-Vu et al., 2010), although they do not directly modulate vigilance states. This further highlights the complexity of the mamalian brain, and the regulation of sleep and waking.

Dopamine and psychostimulants

The role for DA in sleep-wake regulation was initially believed to be of minor importance since the neurotransmitter DA showed only limited alterations across the sleep wake cycle, as observed in freely moving cats (Miller et al., 1983; Steinfels et al., 1983; Trulson and Preussler, 1984). However, recent evidence from rats revealed how extracellular DA levels in the medial prefrontal cortex (mPFC) and part of the ventral striatum known as the nucleus accumbens (NAc), are high during wakefulness and REM sleep, but significantly lower during NREM sleep (Léna et al., 2005). Similarly, Dahan et al., could show that dopaminergic neurons originating from the VTA of the BG show increased burst-firing during REM sleep (Dahan et al., 2007). The BG plays a crucial role for sleep-wake regulation by integrating signals from the cortex, thalamus, amygdala and the midbrain dopaminergic neurons, making it a central part of the ARAS (Monti and Monti, 2007; Lazarus et al., 2012; 2013) (Figure 6). Dopaminergic neurons originating from the VTA innervate the striatum, where inhibitory G-protein coupled dopamine D_2 receptors co-localize with adenosine A_{2A} receptors (Fuxe et al., 2005). Such co-localization indicates interplay between the dopaminergic and adenosinergic systems, and may suggest a pathway for DA in modulating sleep and wakefulness. Although the exact origin of adenosine neurons projecting to the striatum is unknown, the sleep promoting effects following activation of A_1 and A_{2A} receptors, are well established (Porkka-Heiskanen et al., 1997; Basheer et al., 2004). Adenosine levels in the brain are known to increase during waking and decline during sleep (Porkka-Heiskanen et al., 1997). Also, the stimulating effects of the methylxanthine caffeine, are mediated via the adenosine system, where caffeine acts as a antagonist on A_1 and A_{2A} receptors. Recent evidence suggest that the stimulatory effects of caffeine are primarily facilitated by adenosine A_{2A} receptors (Huang et al., 2005; Rétey et al., 2007; Landolt, 2008; Bodenmann et al., 2012). Human studies have revealed that caffeine attenuates homeostatic EEG markers in NREM sleep, as observed in baseline sleep when compared to placebo, but also when the sleep homeostat is challenged by sleep deprivation (Landolt et al., 1995; 2004). Moreover and as previously mentioned, the

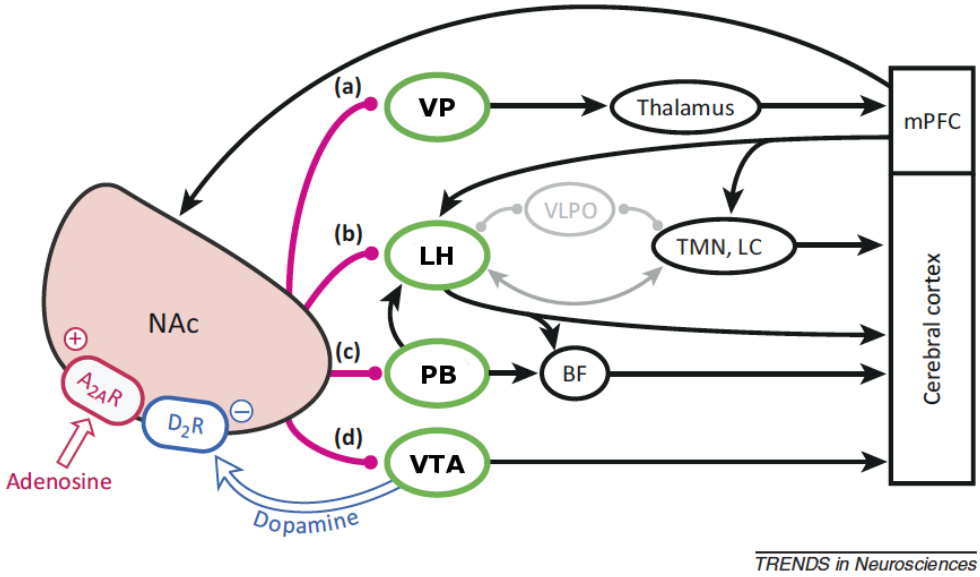
response to caffeine can be modulated by a distinct 1083T>C polymorphism of the ADORA2A gene (Rétey et al., 2007; Bodenmann et al., 2012).

Via the co-localization of A_{2A}-D_2 receptors, adenosine and DA are suggested to have reciprocal innervation of the NAc of the ventral striatum (Figure 6) (Lazarus et al., 2012). The NAc have distinct inhibitory GABAergic connections to some of the main nuclei of the ARAS. Specifically, the NAc can inhibit the ventral palidum (VP) (an area of the BG involved in addiction) and thereby parts of the thalamus and the mPFC, a pathway, which is important for cognition and emotional processes, sensitive to sleep need (Muzur et al., 2002; Chuah et al., 2006) (Figure 6a). A second pathway inhibits ORX neurons of the LH (Figure 6b). Indeed, ORX neurons play a key role in maintaining wakefulness, and selective loss of these neurons results in narcolepsy (Chemelli et al., 1999). Furthermore, the LH contains glutamatergic neurons and has major projections to the BF and cerebral cortex. The LH is also inhibited by the VLPO and shows reciprocal connections to the TMN and LC (Yoshida et al., 2006; Sano and Yokoi, 2007) (Figure 6b). A third pathway inhibits the parabrachial nucleus (PB), the BF and the cerebral cortex, a pathway also involved in maintaining wakefulness and cognitive performance (Usuda et al., 1998; Li et al., 2012) (Figure 6c). The fourth pathway illustrates the feedback loop between the NAc and the dopaminergic neurons of the VTA (Figure 6d). This feedback loop highlights a central role for dopaminergic neurons in regulating sleep and wakefulness (Lazarus et al., 2012; 2013). According to this model by Lazarus and colleagues, activation of dopaminergic neurons in the VTA activate inhibitory dopamine D_2 receptors of the NAc, leading to a reduction of GABAergic inhibition of the four activating pathways (Figure 6 a-d). This in turn will enhance wakefulness and suppress sleep. On the other hand, activation of excitatory A_{2A} receptors on the NAc promotes the inhibition of the four activating pathways and thus promotes sleep.

Evidence for the significance of the VTA projections in modulating sleep and wake, as proposed in the model of Lazarus, is slowly increasing. Dopamine transporter (DAT) knock-out mice show enhanced wakefulness and are hypersensitive to caffeine (Wisor et al., 2001). Indeed, the DAT is highly expressed in the striatum and VTA, and it can be speculated that the removal of DAT causes dramatic changes to the inputs of the NAc making the sleep-wake regulation more dependent on adenosine. On the other hand, the elimination of dopamine D_2 receptors in mice reduces wakefulness and enhances REM and NREM sleep (Qu et al., 2010). Furthermore, the activation of dopamine D_2-type receptors in the NAc

enhances wakefulness, while the blockage of these same NAc D_2 receptors enhances sleep (Barik and de Beaurepaire, 2005).

Figure 6: Incorporation of the NAc of the ventral striatum into the ARAS.



The figure illustrates four activating pathways (a-d) promoting wakefulness. The NAc is a central cluster with inhibitory innervations (magenta round-headed lines) to all four pathways. The model proposes a central role for the VTA given its capability to feedback onto the NAc. As result, wakefulness is promoted upon activation of the VTA. Adapted from Lazarus et al. 2012.
Plus sign (+) represents excitatory receptors; minus sign (-) represents inhibitory receptors. Arrows represent excitatory synapses; round-headed lines represent inhibitory synapses; bars with both symbols represent reciprocal excitatory (arrows) and inhibitory (round-headed) connections. $A_{2A}R$: Adenosine A_{2A} receptors, BF: Basal forebrain, D_2R: Dopamine D_2 receptors, LC: Locus coeruleus, LH: Lateral hypothalamus, mPFC: Medial prefrontal cortex, NAc: Nucleus accumbens, PB: Parabrachial nucleus, TMN: Hypothalamic tuberomammillary nucleus, VLPO: Ventrolateral preoptic nucleus, VP: Ventral palidum, VTA: Ventral tegmental area.

Human brain imaging data corroborate these preclinical findings in mice. Dopamine D_2 receptors have been reported to be down-regulated in the NAc and ventral striatum following sleep deprivation, a decrease that is correlated with reduced cognitive performance (Volkow et al., 2008; 2009b; 2012). Nevertheless, it is very likely that other dopaminergic projections also play a role in sleep-wake regulation. Indeed, selective lesions to the dopaminergic neurons of the ventral periaqueductal gray (vPAG) reduce wakefulness and enhance sleep (Lu et al., 2006; Ueno et al., 2012).

Other region-unspecific evidence for an involvement of DA in sleep-wake regulation arises from studies investigating the potent psychostimulant modafinil

(also known as 2-[(Diphenylmethyl) sulfinyl]acetamide). Modafinil is used as the first-line of treatment for excessive daytime sleepiness in narcoleptic patients, but it is also administered to relieve symptoms of sleepiness in shift workers and apnea patients. Off label use is reported among patients suffering from multiple sclerosis, attention deficit/hyperactive disorder (ADHD), Parkinson's disease and schizophrenia (reviewed in (Minzenberg and Carter, 2008)). Similar to caffeine, modafinil is reported to be used among healthy people to enhance cognition and reduce sleep need (Wesensten, 2006; Maher, 2008). Although modafinil clearly enhances wakefulness, the actual molecular mechanisms are still debated. *In vivo* animal studies revealed that modafinil interferes with both the DAT and the norepinephrine transporter (NET) (Madras et al., 2006; Mitchell et al., 2008). On the other hand, the wake promoting effects of modafinil, as well as other DA dependent stimulants, are abolished in DAT knock-out mice (Giros et al., 1996; Wisor et al., 2001). Data from human positron emission tomography (PET) imaging revealed that modafinil appears to inhibit the DAT and increase the levels of DA in the striatum and NAc. Overall, this suggests a mode of action for modafinil similar to that of amphetamine (Volkow et al., 2009a), although it is likely that modafinil interferes with both dopaminergic and noradrenergic neurotransmission. It is therefore not surprising that the wake promoting actions of modafinil differ in key areas from those of caffeine. Whereas caffeine is known to interfere with homeostatic markers of sleep need such as SWA and SWS, the administration of modafinil shows no such effects, not even when challenging sleep-wake regulation by sleep deprivation (Saletu et al., 1989; Bodenmann et al., 2009b; Bodenmann and Landolt, 2010). Nevertheless, modafinil modulate the sleep EEG, but in a *COMT* genotype dependent manner (Bodenmann and Landolt, 2010). Indeed, the Val158Met polymorphism of *COMT* is known to affect enzymatic activity (Akil et al., 2003; Chen et al., 2004). The COMT is responsible for degradation of catecholamines and may play a key role in regulating dopaminergic neurotransmission in the prefrontal cortex, where the DAT show only very limited expression (Sesack et al., 1998; Morón et al., 2002).

In summary, DA may have an important and central role to play in regulating sleep and wakefulness. Also, the psychostimulant effects of caffeine and modafinil may be facilitated directly or indirectly by dopaminergic neurotransmission. Nevertheless, the exact molecular mechanisms of how DA promotes wakefulness and facilitates the effects of psychostimulants are not yet fully understood.

Synaptic dopaminergic neurotransmission

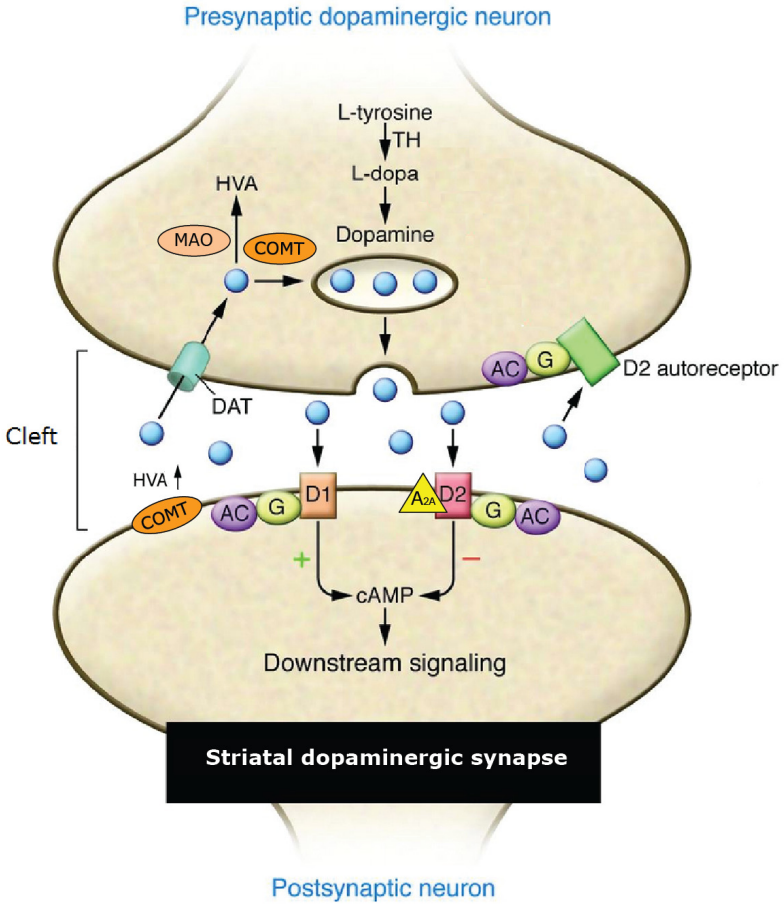
Dopaminegic neurotransmission relies on several key steps including: DA synthesis, DA receptor activation, DA reuptake and DA degradation. DA is synthesized from tyrosine in two steps. Initially tyrosine is converted into L-dopa by L-tyrosine hydroxylase (TH), which is then converted into DA by L-aromatic amino acid decarboxylase (Figure 7). Following presynaptic vesicular storage, DA can be released into the synaptic cleft following an action potential and the corresponding membrane depolarization. Once DA is released into the synaptic cleft, it will bind to dopamine D_1 and D_2 type G-protein coupled receptors resulting in downstream signaling. A key protein in regulation DA neurotransmission is the DAT which is responsible the reuptake of DA into the presynaptic neuron (Giros and Caron, 1993) (Figure 7). It is well established that the reuptake of DA by the DAT involves sequential binding and co-transport of two Na^+ ions and one Cl^- ion per DA molecule. As a result, the actual driving force for DAT-mediated DA reuptake is the ion concentration gradient generated by the Na^+/K^+-ATPase on the presynaptic membrane (Sonders et al., 1997; Torres et al., 2003). This highlights how the activity of the DAT also depends on the presynaptic membrane potential.

DAT expression in the brain is region-specific. While the DAT show only limited expression in the prefrontal cortex, it is highly expressed in the striatum. Specifically, the DAT is expressed in the VTA and SN of the striatum, areas associated with the nigrostriatal, mesolimbic, and mesocortical dopaminergic pathways (Scatton et al., 1985; Ciliax et al., 1999; Lewis et al., 2001; Torres et al., 2003). Additionally, the DAT is believed to be the rate limiting protein for synaptic removal of DA in the striatum (Jones et al., 1998; Benoit-Marand et al., 2000). The human gene encoding DAT (*DAT1*) is located on chromosome 5 and contains a 3'-UTR VNTR polymorphism (SNP-ID: rs28363170). This 40-bp sequence may occur in forms from 3 to 11 repeats, yet the presence of 9 or 10 repeats is most common (Vandenbergh et al., 1992). Although the functional significance of *DAT1* is still debated, accumulating evidence in young healthy adults indicates that 10-repeat allele homozygotes (10R/10R) have 15-20 % reduced DAT availability in the striatum when compared to hetero- and homozygous 9-repeat allele (9R) carriers (Jacobsen et al., 2000; van Dyck et al., 2005; van de Giessen et al., 2009; Costa et al., 2011; Spencer et al., 2013).

Other key proteins involved in the regulation of dopaminergic neurotransmission

include the enzymes COMT, alongside with monoamine oxidase (MAO) A and B - enzymes which degrade DA via several steps into homovanilic acid (HVA) (Figure 7). MAO is typically bound to the outer membrane of mitochondria where they can catalyze monoamines by removing an amine group via oxidative deamination (Youdim et al., 2006) (Figure 7). As previously discussed, COMT is responsible for degradation of catecholamines by catalyzing the transfer of a methyl group onto the catecholamine. Two distinct isoforms of COMT exists: Membrane-bound COMT (MB-COMT); and soluble COMT (S-COMT); where the MB-COMT is the most abundant isoform in the human brain (Tunbridge et al., 2006) (Figure 7). Given the low expression of DAT in the prefrontal cortex, COMT has been suggested to play a key role in regulating dopaminergic neurotransmission in this brain-region (Sesack et al., 1998; Morón et al., 2002).

Figure 7: Simplified illustration of a striatal dopaminergic synapse.



The figure illustrates the synthesis of DA from tyrosine by L-tyrosine hydroxylase (TH). Following an action potential, vesicular stored DA is released into the synaptic cleft where it is rapidly taken up by DAT for recycling, or degraded by COMT and MAO into homovanilic acid (HVA). Adapted from Tunbridge et al., 2006; Blackstone, 2009.

The *COMT* gene, unlike *DAT1*, contains a well-established functional polymorphism (Val158Met, SNP-ID: rs4680) of MB-COMT, which leads to a Val-to-Met amino acid substitution in the COMT protein. The polymorphism reduces the thermo-stability of the enzyme, which leads to a 3-4 fold decrease in enzymatic activity in homozygous Met-allele carriers when compared to Val (Akil et al., 2003; Chen et al., 2004). The COMT gene is located on chromosome 22.

DA does not only play a critical role in the striatum and BG. As a widely projecting neurotransmitter, DA is also involved in the regulation of frontal network dependent cognitive processes, specifically in the mPFC and frontal lobe. Frontal processing has been suggested to be dependent on dopaminergic levels according to an inverted-U-shape relationship, which may be modulated by COMT (Tunbridge et al., 2006). According to this paradigm, any "homeostatic" challenge (e.g. sleep restriction, cognitive load etc.) will cause a shift in cortical DA levels and thus also, cortical function. Under this paradigm, a number of studies have associated the highly active Val allele of COMT with poorer working memory (Malhotra et al., 2002; Blasi et al., 2005; Bodenmann et al., 2009a) and a number of psychiatric disorders including schizophrenia and bipolar disorder (see Tunbridge et al., 2006 for review). Furthermore, Dauvilliers and colleagues demonstrated that the COMT Val158Met polymorphism affects daytime symptoms of narcolepsy in a gender dependent manner (Dauvilliers et al., 2001).

Based on the proposed role for DA, and in particular for DAT and COMT, we studied the two polymorphisms (*DAT1*, rs28363170 and *COMT*, rs4680) to investigate the role of DAT and COMT on the daily and habitual regulation of sleep and wakefulness, in a large-scale, 4 week, actigraphy-derived rest-activity study, including 65 men and 45 women (Chapter 2). Based on the findings in the actigraphy study, we further investigated 57 subjects for the specific effects of DAT on sleep-wake regulation, in a controlled sleep deprivation study, which included the administration of caffeine (2 x 200 mg) and modafinil (2 x 100 mg) to a subgroup of participants (Chapter 3).

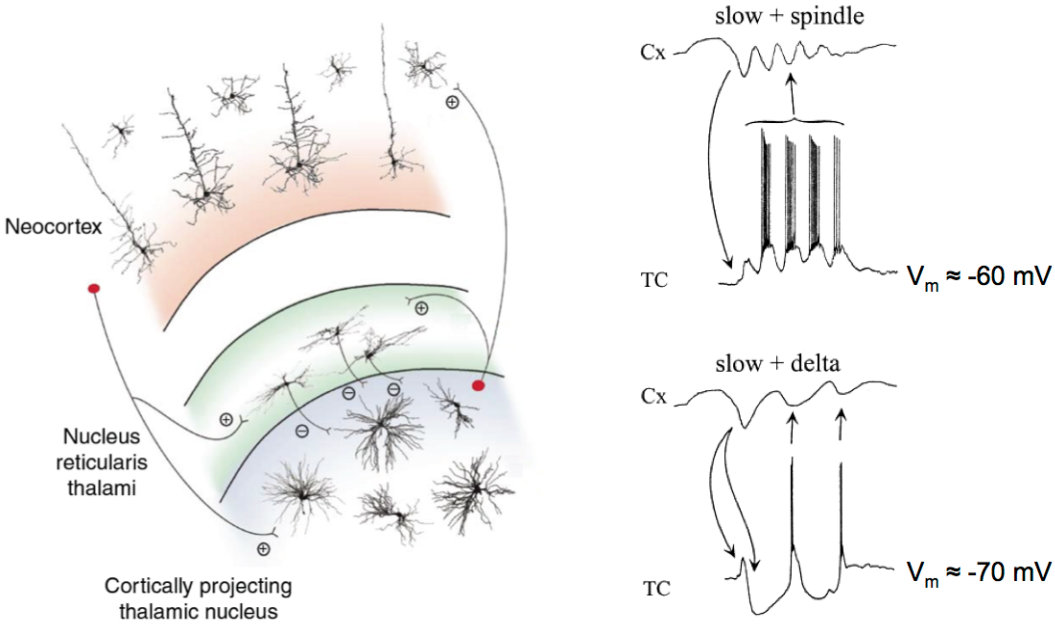
Corticothalamic regulation of brain oscillations

The three main oscillations observed in the human sleep EEG - slow oscillations, delta waves and spindles, - are hallmarks of neuronal interactions between the thalamus and cortex. The thalamus can be divided into thalamocortical and reticular

cells. The thalamocortical cells are relay neurons exhibiting excitatory glutamatergic projections to the cortex and to the reticular thalamic cells (Figure 8). On the other hand, the reticular cells only have inhibitory connections to the thalamocortical cells releasing GABA. Finally, cortical pyramidal cells are capable of innervating both reticular and thalamocortical cells, via glutamatergic excitatory connections. Combined, the thalamus and cortex form a loop capable of generating the major EEG oscillations observed in the human EEG (Amzica and da Silva, 2012) (Figure 8).

The neuronal firing patterns of the thalamus depend on the vigilance state. At the transition from wakefulness to NREM sleep, inputs from the ARAS are reduced and the thalamocortical cells enter a burst-pause firing mode with long periods of hyperpolarization, which inhibits the transmission of signals through the thalamus to the cortex (Moruzzi and Magoun, 1949; Steriade, 1970). In the beginning of NREM sleep when the homeostatic drive for sleep is high, the thalamocortical cells are hyperpolarized and exhibit resting membrane potentials (V_m) of around -65 mV to -70 mV (Figure 8). This triggers delta oscillations, an intrinsic firing mode of the thalamocortical network characterized by two hyperpolarization-activated inward currents (McCormick and Pape, 1990; Steriade et al., 1991). Indeed, these NREM delta waves are the main source of SWA, the classic EEG marker of sleep homeostasis (Figure 4). Spindles, on the other hand, are generated at more depolarized membrane potentials in the thalamus at around -60 mV (Figure 4). Spindles themselves arise from the reticular thalamic neurons transmitting IPSPs to the thalamocortical relay neurons. This in turn triggers rhythmic burst-pause spindle oscillations in the thalamocortical cells, which is transmitted to the cortex (Steriade et al., 1990b). Meanwhile, the slow oscillations which were discovered by Steriade and colleagues (Steriade et al., 1993a), are primarily cortically generated and can occur at different thalamic resting membrane potentials. They consist of prolonged depolarized up-states associated with neuronal firing. The slow oscillation can group together periods of sleep spindles and delta waves during their up- (depolarization) and down- (hyperpolarization) states. They are also observed in the human EEG and show peak frequency between 0.7 - 0.8 Hz (Achermann and Borbély, 1997; Crunelli and Hughes, 2010). Slow oscillations are seen during all stages of NREM sleep, although their actual generation is still debated. Slow oscillations have a strong cortical component and are observed in the cortex even without thalamic inputs (Sanchez-Vives and McCormick, 2000; Crunelli and Hughes, 2010), yet vanish when the thalamus is isolated from the cortex (Riedner et al., 2011).

Figure 8: The cortico-thalamo-cortical system and the generation of EEG oscillations



The thalamus is divided into reticular and thalamocortical neurons. Excitatory cortical pyramidal cells innervate both reticular and thalamocortical neurons. Similarly, thalamocortical neurons have excitatory innervation of both cortex and reticular thalamic neurons, whereas the reticular cells have inhibitory interneuronal connections (GABAergic) to the thalamocortical cells. These cells make up the so-called thalamocortical loop, + and − indicate excitatory and inhibitory synapses, respectively. The thalamus plays an important role in generating some of the major EEG hallmarks of sleep, namely delta waves and spindles. Furthermore the thalamus facilitates the slow oscillations. The resting membrane potential of the thalamus during NREM sleep leads to the generation of either delta waves (V_m ~ -70 mV) or spindles (V_m ~ -60 mV). Adapted from Crunelli and Hughes 2010 and Steriade et al., 2003.

Slow oscillations have been characterised as travelling waves (Massimini et al., 2004) and EEG-based source modelling has located their main origin in the cingulate gyrus and left insula (Murphy et al., 2009; Riedner et al., 2011). MRI and PET imaging further linked the origin to areas around the medial and inferior frontal cortex, precuneus and posterior cingulate (Dang-Vu et al., 2005; 2008). Finally the large slow waves (> 140 μV) were associated with activity in the parahippocampal gyrus cerebellum and brain stem (Mascetti et al., 2011). Group I metabotropic glutamate receptors (mGluR), which will be extensively covered in following sections, have also been linked to the regulation of slow oscillations in NREM sleep (Hughes et al., 2002; Blethyn et al., 2006) as well as theta activity during waking (Cobb et al., 2000).

Interestingly, slow oscillations have been linked to neuronal plasticity and memory processing (Steriade and Timofeev, 2003). Specifically, Marshall and colleagues

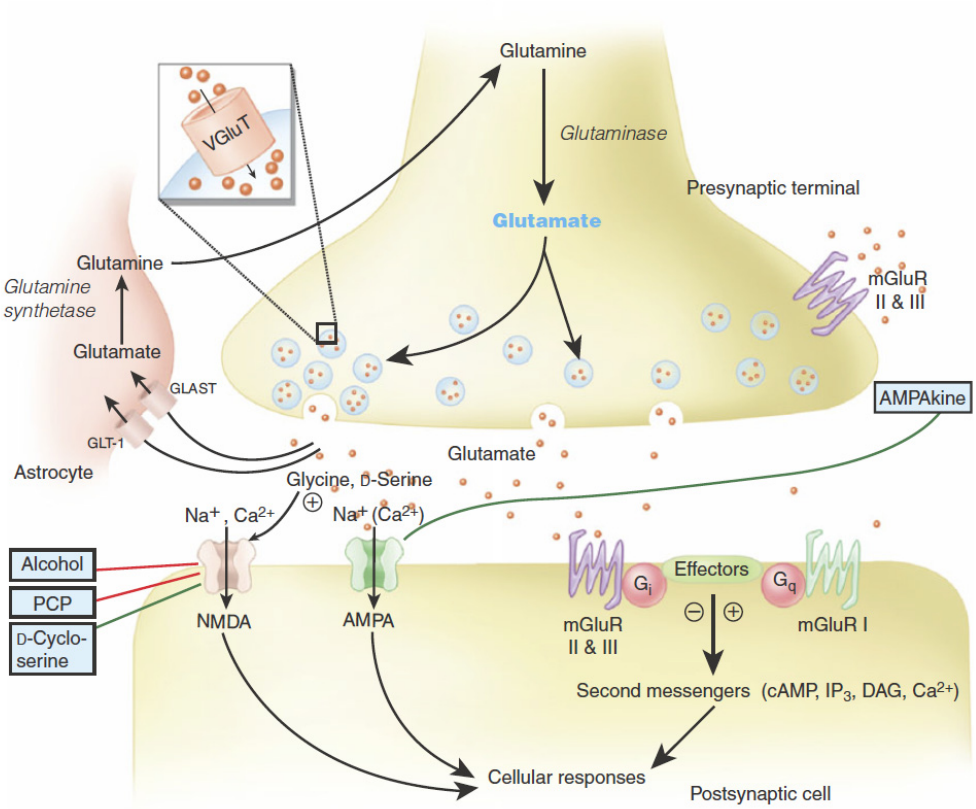
could show that the induction of oscillating potential fields to the humans scalp in the slow oscillating range (0.75 Hz), could enhance memory recall of word-pairs following a night of sleep (Marshall et al., 2006), while stimulation at 5 Hz reduced word-pair memory performance (Marshall et al., 2011). Collectively, these findings may show an interesting aspect of slow oscillations that are in need of further investigation.

Glutamatergic signalling in the CNS

Synaptic plasticity associated with learning and memory is related to glutamatergic neurotransmission in general, and to the availability and activation of ionotropic glutamate receptors, such as a-amino-3-hydroxy-5- methyl-4-isoxazole propionic acid receptors (AMPARs) and N-methyl-d-aspartate receptors (NMDARs) in particular (Malenka and Bear, 2004; Kauer and Malenka, 2007). Indeed, glutamate plays a key role in the human CNS, where it is expressed in high concentration and considered the major excitatory neurotransmitter. Glutamate is synthesised from glutamine and stored in vesicles by the vesicular glutamate transporter (VGluT), a subtype of excitatory amino acid reuptake transporters (EAAT) (Figure 9). Following electrical stimulation, glutamate is released into the synaptic cleft in a Ca^{2+} dependent manner, where it can act via ionotropic and metabotropic glutamate receptors, post- or pre-synaptically. The synaptic actions of glutamate are mainly concluded by uptake into glial cells by different classes of EAATs, such as GLT-1 and GLAST. In glia, glutamate is converted into glutamine for recycling (Nestler et al., 2009b). Group I mGluRs (mGluR1 & mGluR5) activate phosporlipase C, which catalyses the second messenger's inositol triphosphate (IP3) and diacyl glycerol (DAG). This enhances downstream signal amplification and protein synthesis (Nelson and Cox, 2008) thereby indirectly controlling NMDAR and AMPAR expression (Oliet et al., 1997; Collingridge et al., 2004). Group I mGluRs are found on astrocytes and neurons, and are mainly post- and extra-synaptic (van den Pol et al., 1995; Lujan et al., 1996; Fellin et al., 2004) (Figure 9). Other mGluR types include group II (mGluR2 and mGluR3) and group III (mGluR4, mGluR6, mGluR7 and mGluR8) mGluRs, which are typically presynaptic and modulate neurotransmitter release (Figure 9). Group II and III mGluRs inhibit adenyl cyclase and reduce neuronal transmission. An important function of Group I mGLuRs is their involvement in neuronal plasticity, LTD, and to some extent LTP (Lu et al., 1997; Ayala et al., 2009; Izumi and Zorumski, 2012). Although other receptors may also be involved along side the mGluRs, they have specifically been linked to plastic

processes in the neocortex, striatum, nucleus accumbens and hippocampus (reviewed by Anwyl, 2009). Indeed, several studies have linked sleep-wake regulation to alterations in glutamate and LTP-processes.

Figure 9: Simplified illustration of a glutamatergic synapse.



Following the synthesis of glutamate from glutamine, glutamate is stored in vesicles and can be released in response to an action potential. Following release, glutamate may interact with ionotropic or metabotropic glutamate receptors, pre-, post-, or extra-synaptically. Following the uptake of glutamate into glial cells, glutamate is converted into glutamine and is transported back into the presynpase for recycling. From (Nestler et al., 2009b).

Glutamate levels in the frontal cortex, measured in freely moving rats, were shown to increase progressively during waking and REM sleep, but decreased during NREM sleep (Dash et al., 2009). Microarray analysis comparing mRNA levels in sleeping and awake rats revealed that: the synthesis of glutamate; the clustering of glutamate receptors (Homer/Vesl & NARP transcription levels); and markers of LTP (Arc, BDNF & NGFI-A transcription levels); were higher during wakefulness than sleep (Cirelli et al., 2004). The study also revealed that mRNA transcripts related to the protein translational machinery (EEF2 & eIF4 transcription levels), and several other elements related to myelin maintenance and membrane trafficking, were increased during sleep (Cirelli et al., 2004). This supports an active role for sleep in

protein synthesis and neural plasticity. Additionally, Vyazovskiy and colleagues could show a reduction of AMPARs during sleep in rats (Vyazovskiy et al., 2008a). Furthermore, wakefulness was associated with an enhanced phosphorylation of AMPARs and CAMKII, suggesting a form of progressive potentiation (Vyazovskiy et al., 2008a). Combined with an enhancement of evoked cortical potentials during waking, these findings can be interpreted as synaptic potentiation or LTP, occurring during wakefulness and counterbalanced during sleep (Vyazovskiy et al., 2008a; Huber et al., 2012).

The mGluR5 are especially interesting as they have been associated with a number of CNS functions including anxiety, memory, autism, learning, pain perception, depression and addiction (Swanson et al., 2005; Barker et al., 2006; Dölen et al., 2007; Xu et al., 2009; Deschwanden et al., 2011; Jung et al., 2011; Hulka et al., 2013). Although these effects may seem disassociated, they are all likely to be linked to plastic neuronal processes. Evidence also suggests that the mGluR5 receptors may be specifically associated with sleep-wake regulation. Several brain regions associated with the ARAS (e.g. the ventromedial prefrontal cortex, BF, insula, anterior cingulate cortex, striatum, parahippocampal gyrus, precuneus, etc.) are brain regions where mGluR5 are preferentially expressed (Ametamey et al., 2007; Gasparini et al., 2008). Furthermore, and as previously explained, the mGluR5 are known to play an important role in shaping NREM EEG slow oscillation (Hughes et al., 2002; Blethyn et al., 2006). Additionally, a distinct interaction between DA D_2, adenosine A_{2A} and mGluR5 receptors have been described specifically in the striatum (Ferré et al., 2007). Finally, given how G-protein coupled metabotropic receptors are tightly regulated and provide a powerful mechanism of signal amplification (Nelson and Cox, 2008), even minor changes may be linked to relevant physiological consequences. Taken together, convening evidence supports a role for the mGluR5 in modulating sleep-wake regulation.

Today, it is possible to accurately quantify the mGluR5 *in vivo* by molecular PET imaging with the highly selective non-competitive mGluR5 antagonist ^{11}C-ABP688 (Ametamey et al., 2006). In line with pre-clinical knowledge (Romano et al., 1995), the availability of mGluR5 was found to be high in the anterior cingulate, medial temporal lobe, amygdala and striatum; whereas white matter and the cerebellum revealed only low levels of mGluR5 (Ametamey et al., 2007). Based on the associations between neuronal plasticity, sleep-wake regulation and mGluR5 receptor expression; in chapter 4 and 5, we investigated if mGluR5 may be linked to

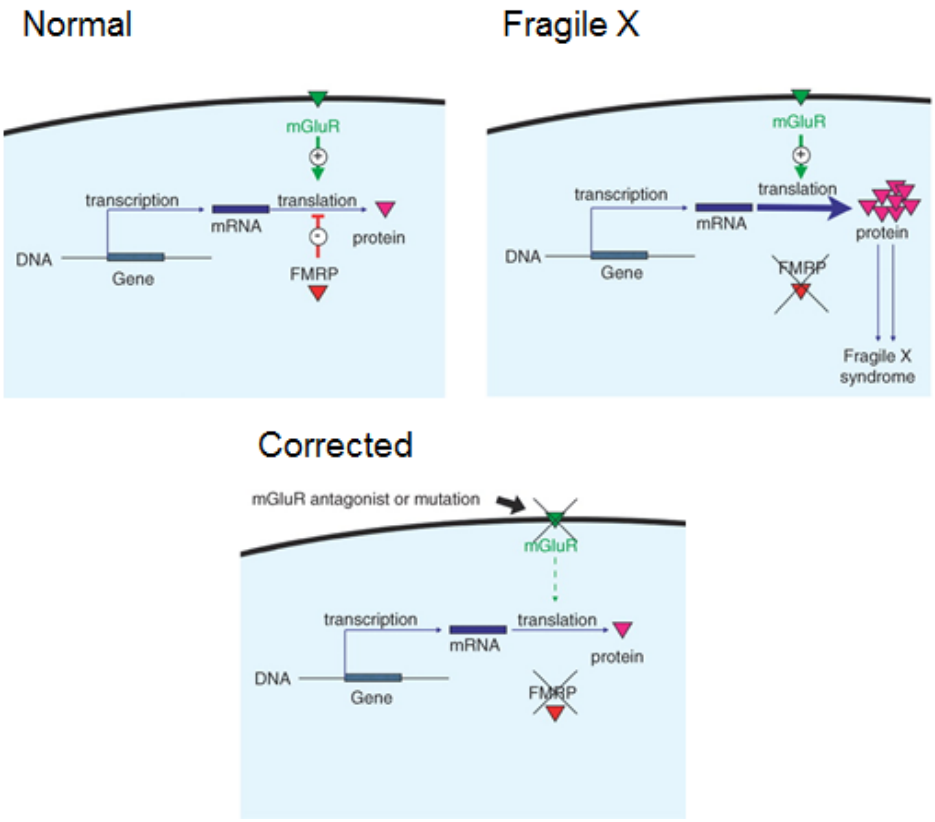
sleep-wake regulation in healthy adult men. We used the PET ligand ^{11}C-ABP688 to quantify mGluR5 availability after 9 and 33 hours of wakefulness.

Sleep regulation and the mGluR5 – FMR1 interaction

The most widespread single-gene cause of autism is the fragile X syndrome (FXS), triggered by a 5' UTR $(CGG)_n$ repeat expansion in the Fragile X mental retardation 1 (*FMR1*) gene, located on the X chromosome. Following a CGG triplet expansion above 200 repeats, the DNA is hypermethylated and the FMR1 gene completely silenced (Cummings and Zoghbi, 2000). Although such triplet repeats (often denoted as microsatellites) are actually common in the human genome and are observed approximately every 2 kb of DNA (Barker, 2002), they are rarely associated with any disease. However, because of the large (> 200) repeat sequence, it is likely (although not yet conclusively shown) that slipped-strand mispairing interferes with normal DNA replication and causes the observed polymorphic transmission of CGG repeats across generations (Pearson et al., 2005). The silencing of the *FMR1* gene results in the complete loss of the fragile X mental retardation protein (FMRP) (Robertson, 2005).

The FMRP is a RNA binding protein expressed in the brain, which is associated with polyribosomes and regulates neuronal mRNA trafficking, translation and synthesis at synapses (Bagni and Greenough, 2005; Ben A Oostra and Willemsen, 2009). The FMRP is important for synaptic plasticity, LTP and LTD in neuronal cell bodies and dendritic spines. Specifically, the FMRP is believed to inhibit downstream signaling from group I mGluRs thereby maintaining translation at an optimal level. The loss of FMRP leads to an uncontrollable activation of mGLuR1 and mGLuR5, thereby causing of FXS. This concept is known as the "mGluR theory of FXS" (Bear et al., 2004; Dölen and Bear, 2008) (Figure 10). In the absence of FMRP, the initiation of protein synthesis from mGluRs is no longer sufficiently regulated, and excessive AMPAR internalization and enhanced LTD is observed (Bassell and Warren, 2008). As such, the loss of FMRP is associated with long, thin and immature dendritic spines (Irwin et al., 2001; Nimchinsky et al., 2001). Strong support of the mGluR theory of FXS came from the treatment of *FMR1* knock-out mice with selective mGluR5 inhibitors, which were reported to rescue the FXS phenotype (Dölen et al., 2007; Michalon et al., 2012) (Figure 10). Taken together, these findings suggest a strong interaction between mGluR5 and FMRP.

Figure 10: The mGluR theory of fragile X syndrome



A simplified scheme of how mGluR5 and FMRP regulates protein translation. In normal healthy individuals, mGluR5 and FMRP together maintain protein translation at an optimal level. Among fragile X patients, the loss of FMRP leads to an up-regulation of protein translation, causing FXS. The application of a mGluR5 antagonist to FXS patients reduce protein translation initiation and thereby conceptually counterbalance the FXS phenotype. From Dölen and Bear, 2008.

The neuronal changes associated with FXS causes severe cognitive deficits in human FXS patients, a condition associated with severely reduced intelligence (low IQ ~ 40). Specifically, FXS in humans has been associated with reduced working and short-term memory, reduced executive function, disturbed sleep, anxiety and obsessive-compulsive disorder (Penagarikano et al., 2007). Furthermore, spatial features, such as an elongated face and large ears, are commonly observed. The loss of FMRP and its impact on sleep, has been extensively studied in animal models. Brain FMRP levels in drosophila have been shown to increase with time awake, and to be inversely linked to sleep duration (Bushey et al., 2009). Furthermore, overexpression of FMRP in the fly model revealed reduced neuronal plasticity in response to sleep deprivation and an overall reduced sleep duration (Bushey et al., 2011). Additionally, the mGluR5 has been linked to synaptic processes and to the regulation of sleep and wakefulness in other studies. For

instance, an associations between mGluR5 and the immediate early gene Homer1a (Ronesi et al., 2012); a well defined marker of sleep need (Maret et al., 2007) has been reported.

A mild form of FXS is observed in so-called “premutation carriers”. As defined by The American College of Medical Genetics, the premutation is diagnosed when the number of CGG repeats in the FMR1 gene is are between 54 – 199 (Maddalena et al., 2001). This mild form of FXS has been associated with fragile X tremor ataxia syndrome (FXTAS) and premature ovarian failure (Bourgeois et al., 2009). The premutation range is associated with increased FMR1 mRNA levels, yet decreased FMRP expression (Tassone et al., 2000; Allen et al., 2004; Peprah et al., 2009). Furthermore, specific AGG interruptions in the $(CGG)_n$ sequence exists, which increase repeat stability and reduce the risk of maternal premutation carriers transmitting a full mutation to their offspring (Yrigollen et al., 2012). Nevertheless, the AGG interruptions have not been shown to affect FMRP expression levels in premutation carriers (Yrigollen et al., 2011). Among healthy individuals, very little is known about how *FMRP* expression is regulated, or whether the number of *FMR1* CGG repeats plays a significant role. Studies examining both premutation and healthy carriers have shown an increase in *FMR1* transcription levels and a reduction in FMRP levels with increasing CGG repeat number. However, within the healthy CGG repeat range alone, no association was found (Allen et al., 2004; Peprah et al., 2009).

Based on the association between the *FMR1* CGG expansion, mGluR5, and sleep-wake regulation observed in animal models, we hypothesized that these proteins would also affect healthy human sleep and wakefulness. This potential association is investigated in chapter 5.

Main research aims of the thesis

As mentioned in the previous sections, the main research goals of this thesis are to investigate the roles of the dopaminergic and glutamatergic neurotransmitter systems in regulating wakefulness and sleep.

In Chapter 2 and 3, two polymorphisms of the genes coding for the DAT and COMT (*DAT1* - rs28363170 and *COMT* - rs4680, respectively) will be investigated to elaborate on the effects of DA, in modulating sleep and waking. Specifically, Chapter

2 will investigate the role of DAT and COMT on daily actigraphy-derived rest-activity patters across a 4-week period among 65 men and 45 women. In Chapter 3, the specific role of DAT will be further examined in a subgroup of 57 participants who underwent a controlled sleep deprivation protocol, which included the administration of caffeine (2 x 200 mg, n=16) and modafinil (2 x 100 mg, n=22). Nocturnal EEG recordings was analysed in baseline and recovery sleep and the effects of the *DAT1* genotype, caffeine, and modafinil investigated.

In Chapter 4 and 5, the role of glutamate in sleep-wake regulation is examined. 26 healthy men underwent two PET imaging sessions following 9 and 33 hours of sustained wakefulness. The quantification of mGluR5 availability was achieved by using the highly selective non-competitive radio-ligand ^{11}C-ABP688. Chapter 4 will examine how sleep deprivation affects the availability of mGluR5 and its association to subjective sleepiness. In Chapter 5, the interactions between NREM EEG oscillations and mGluR5 will be investigated and combined with genetic estimates of FMRP levels and their role in modulating the mGluR5 availability.

Finally, the “Concluding remarks” in Chapter 6, will discuss and elaborate, on some of the most important findings of this thesis.

Chapter 2

Genetic polymorphisms of *DAT1* and *COMT* differently affect actigraphy-derived sleep-wake cycles in humans

Amandine Valomon[1,2], Sebastian C. Holst[1,2], Valérie Bachmann[1,2], Antoine U. Viola[1,3], Christina Schmidt[3], Jurian Zürcher[4], Wolfgang Berger[2,4,5], Christian Cajochen[3], and Hans-Peter Landolt[1,2,5]

[1] Institute of Pharmacology and Toxicology, University of Zürich, Switzerland
[2] Zürich Centre for Integrative Human Physiology, University of Zürich, Switzerland
[3] Centre for Chronobiology, Psychiatric Hospital of the University of Basel, Switzerland
[4] Institute of Medical Molecular Genetics, University of Zürich, Switzerland
[5] Neuroscience Center Zürich (ZNZ), University and ETH Zürich, Switzerland

In revision: ***Chronobiology International***

Abstract

Accumulating evidence suggests that dopamine plays a role in sleep-wake regulation. Cerebral dopamine levels are regulated primarily by the dopamine transporter (DAT) in the striatum and by catechol-O-methyl-transferase (COMT) in the prefrontal cortex. We hypothesized that the variable-number-tandem-repeat (VNTR) polymorphism in the 3'-untranslated region of the gene encoding DAT (*DAT1, SLC6A3*; SNP-ID: rs28363170) and the Val158Met polymorphism of *COMT* (SNP-ID: rs4680) differently affect actigraphy-derived rest-activity cycles and sleep estimates in healthy adults (65 men; 45 women; age range: 19-35 years). Daytime sleepiness, continuous motor rest-activity and sleep diary data during roughly 4-weeks were analysed. Nine-repeat (9R) allele carriers of *DAT1* more often reported elevated sleepiness (Epworth sleepiness score ≥ 10) than 10-repeat allele homozygotes (10R/10R) ($p < 0.02$). Moreover, male 9R allele carriers showed higher overall activity, whereas this difference was not present in women ('*DAT1 genotype'* x '*gender'* interaction: $p < 0.005$). Rest-activity patterns did neither differ among *COMT* genotypes. Nevertheless, a significant '*COMT genotype'* x '*type of day'* (workdays *vs.* rest days) interaction for sleep duration was observed ($p = 0.03$). The Val/Val and Met/Met homozygotes habitually prolonged sleep on rest days compared to workdays by more than 30 min, while Val/Met heterozygotes did not significantly extend their sleep (mean difference: 7 min). Moreover, whereas the proportion of women among the genotype groups did not differ, *COMT* genotype affected body-mass-index (BMI), such that Val/Met individuals had lower BMI than the homozygous genotypes ($p < 0.04$). In conclusion, our data support a contribution of genetically-determined differences in cerebral dopaminergic neurotransmission in modulating daytime sleepiness and individual rest-activity profiles, as well as other sleep-associated health characteristics such as the regulation of BMI. The differential effects of *DAT1* and *COMT* polymorphisms may reflect the distinct local expression of the encoded proteins in the brain.

Keywords:

Dopamine, *SLC6A3*, circadian, daytime sleepiness, body-mass-index (BMI)

Introduction

The brain levels of dopamine are regulated in the striatum primarily by dopamine transporter (DAT), which removes dopamine from the synaptic cleft, and in the prefrontal cortex primarily by catechol-O-methyl-transferase (COMT), which degrades dopamine to 3-methoxy-tyramine. Accumulating evidence suggests that dopamine is a prominent regulator of wakefulness and sleep (Monti and Monti, 2007), which are controlled by the intricate interplay of the endogenous circadian clock and a sleep-wake dependent, homeostatic process (Borbély, 1982). Pronounced diurnal fluctuations of extracellular brain dopamine levels across 24 h rest-activity and sleep-wake cycles are well established (Rye and Freeman, 2011). Furthermore, disease states with altered dopaminergic neurotransmission, such as Parkinson's disease (PD) and attention deficit hyperactivity disorder (ADHD), are often associated with disturbed rest-activity profiles and sleep problems (Whitehead et al., 2008; Van Veen et al., 2010). Apart from influencing the circadian clock, dopamine may also play a role in regulating the homeostatic facet of the sleep-wake cycle. Studies in animal models identified wake-active dopaminergic neurons innervating sleep-promoting regions such as the ventro-lateral pre-optic area (Lu et al., 2006; Ueno et al., 2012), and also pharmacologic and clinical observations suggest an important contribution of dopamine to sleep-wake regulation. For example, stimulants like metamphetamine and agonists of dopamine receptors promote wakefulness and motor activity in mice and humans (Wise and Bozarth, 1987; Wisor et al., 2001). Dopamine D_2 receptor knock-out mice exhibit prolonged sleep time when compared to wild-type animals (Qu et al., 2010), while genetically modified mice and flies without functional dopamine transporter (DAT) have increased wakefulness (Giros et al., 1996; Kume et al., 2005). Finally, patients with PD often suffer from rapid-eye-movement (REM) sleep behavior disorder and excessive daytime sleepiness (Rye and Jankovic, 2002; Comella, 2008). Increased motor activity to counteract elevated sleepiness due to reduced prefrontal dopaminergic 'tone' may also be present in patients with ADHD (Cohen-Zion and Ancoli-Israel, 2004; Cortese et al., 2009). Such a pathophysiology may explain why hyperactivity in ADHD can be improved with medications that increase dopamine release (Minzenberg, 2012).

The gene encoding DAT (*DAT1*, *SLC6A3*) exists in different variants in the healthy population. More specifically, it contains a 40-base pair (bp) variable-number-tandem-repeat (VNTR) polymorphism in its 3'-untranslated region (rs28363170),

expressing 3 to 11 repeats, with the presence of 9 and 10 repeats as the most common isoforms (Vandenbergh et al., 1992; Kang et al., 1999). Although VNTRs in general (Nakamura et al., 1998) and the VNTR domain of *DAT1* in particular (Michelhaugh et al., 2001) can influence transcriptional and translational processes, the consequences of this polymorphism on DAT availability in the human striatum are slightly controversial (Heinz et al., 2000; Jacobsen et al., 2000; van Dyck et al., 2005). Nevertheless, more recent brain imaging studies in young adults consistently suggest that the presence of the 9-repeat (9R) allele of *DAT1* increases striatal DAT availability compared to 10-repeat allele homozygotes (10R/10R) (van de Giessen et al., 2009; Costa et al., 2011; Spencer et al., 2013). This polymorphism affects the rebound in deep slow wave sleep after prolonged waking in healthy volunteers (Holst et al., 2012) (see Chapter 3), and the 9R allele has been associated with ADHD in adult patients (Barkley et al., 2006; Franke et al., 2010; Spencer et al., 2013) who typically exhibit increased DAT expression when compared to matched controls (Dougherty et al., 1999; Dresel et al., 2000; Spencer et al., 2005; Krause et al., 2006; Spencer et al., 2013). We, thus, predicted that 9R carriers of *DAT1* may differ from 10R/10R homozygotes and show elevated sleepiness and increased motor activity across wakefulness and sleep, similar to patients with ADHD in comparison to healthy controls (Boonstra et al., 2007; Surman et al., 2009).

In contrast to the effect of the *DAT1* VNTR, the functional impact of the Val158Met polymorphism (rs4680) in the gene encoding COMT is well established. It leads to a Val-to-Met amino acid substitution in the COMT protein, which reduces thermo-stability of the enzyme and drastically decreases enzymatic activity in Met-allele carriers (Akil et al., 2003; Chen et al., 2004). This polymorphism has been associated with various psychiatric disorders (Hosák, 2007), and it modulates individual responses to modafinil in healthy adults after sleep deprivation (Bodenmann et al., 2009b; Bodenmann and Landolt, 2010) and to methylphenidate in children with ADHD (Kereszturi et al., 2008). Here, we aimed at investigating its effect on long-term actigraphy-derived habitual rest-activity profiles in young men and women. Additionally, because several studies reported an association between *COMT* genotype and food intake (Tworoger et al., 2004; Galvão et al., 2012), we investigated whether this polymorphism had an impact on body-mass-index (BMI), which provides another important sleep-associated health outcome measure that differs widely even among healthy individuals.

Materials and Methods

Study participants

One-hundred twenty-nine young healthy males and females were recruited with flyers and on the Internet for participation in sleep studies at the Universities of Zürich and Basel. The study protocols, screening questionnaires, and consent forms were approved by the local ethics committees and conformed to the Declaration of Helsinki.

A general entrance questionnaire was used to gather demographic information including age, gender, BMI, level of education, and to screen for medical diseases. The inclusion criteria consisted of young age (19-35 years), normal BMI (17-29), good subjective sleep quality, absence of neurological or psychiatric disorders, no history of drug abuse, no current medication intake, no excessive consumption of alcohol, no shift work or crossing of >2 time zones within the last 3 months, and no extreme chronotype as assessed with the Münich ChronoType Questionnaire (Roenneberg et al., 2003). Subjective sleepiness was assessed with the Epworth Sleepiness Scale (Johns, 2000). All subjects received monetary compensation for their participation.

Actimetry

All participants completed 3- to 4-week rest-activity recordings at home, including at least 2 weekends, without any behavioral restrictions. During the recording period, they continuously wore an Actiwatch® (Cambridge Neurotechnology, Cambridge, UK) on their non-dominant arm, and filled-in a sleep-wake diary twice per day. A total of 3001 days and nights worth of actigraphic data were recorded, consisting of 19.5 ± 4.4 workdays and 7.7 ± 2.1 rest days (i.e., weekend days) per study participants. The validity of actigraphic recordings for sleep-wake estimation in healthy volunteers and patients is widely accepted (Sadeh, 2011).

The non-parametric circadian rhythm analysis (NPCRA) developed by Van Someren et al. (1999) was used to determine circadian rhythm characteristics (Van Someren et al., 1999). The analysed outcome variables included the inter-daily stability (IS) (i.e., degree of resemblance between activity patterns of each individual), the intra-daily variability (IV) (i.e., fragmentation of periods of activity and rest), the 5-least-active-hours (L5), the 10-most-active-hours (M10) and the relative amplitude (RA) (ratio between M10 and L5). L5 and M10 were determined from the

24-h average curve indicating the mean activity counts of nadir and peak of the day, as well as their time of onset.

For the activity analysis, raw activity counts were exported from the 'Sleep and Activity Analysis 7.23V software' (Cambridge Neurotechnology Ltd, version 5.42). The data of consecutive 30-min bins were averaged over 24 hours in each individual. For the nighttime sleep analysis, the 'Sleep Scoring Algorithm' module of the same software allowed the investigation of several objective, sleep-related variables, including time in bed (i.e., difference between bed time and rise time), sleep and wake times, estimated sleep duration (i.e., difference between sleep time and wake time), sleep latency (i.e., time between bedtime and sleep onset), sleep efficiency (i.e., estimated sleep duration divided by the time in bed), and wakefulness after sleep onset (WASO). The bed and rise times (i.e., times of lights-off and lights-on) were identified and manually set by the same experienced person (Amandine Valomon) in all nights and subjects. The wake/sensitivity threshold was set to "low", showing optimal agreement between actigraphy and polysmonography (PSG) in this study population (Tonetti et al., 2008). The actimetric recordings were combined with the information contained in the sleep-wake diaries, including bed times, get-up times, caffeine consumption, occurrence of naps, use of alarm clock, etc.

Genotyping

Genomic DNA was isolated from 3 ml fresh EDTA-blood (n = 83) (Wizard® Genomic DNA Purification Kit, Promega, Madison, WI) or from saliva (n = 46) (NucleoSpin® Blood Kit, Macherey-Nagel AG, Oensingen, Switzerland). The genotypes of both *DAT1* and *COMT* could be reliably established in 115 study participants.

The *DAT1* genotypes were determined by PCR on an MJ Research PTC-225 thermal cycler (MJ Research/Bio-Rad, Reno, NV). The following primers were used: forward primer, 5'-tgtggtgtagggaacggcctga-3' and reverse primer 5'-cttcctggaggtcacggctcaa-3' (annealing temperature of 67 °C with HOT FIREPol® DNA Polymerase). The 430-480 bp PCR products were then analysed by agarose gel electrophoresis. COMT genotypes were determined using a Taqman® SNP Genotyping Assay (Life Technologies Europe B.V.) and allelic discrimination analysis was performed using the software SDS v2.2.2 (Applied Biosystems, Foster City, CA,

USA). All analyses were replicated at least once for independent confirmation of results.

Five volunteers had rare *DAT1* genotypes (one 10R/7R, two 9R/8R, two 10R/11R) and were excluded from the analyses. Seven subjects were homozygous for the 9-repeat allele. They were combined with the 9R/10R genotypes and referred to as 9R carriers. The allelic distributions of *DAT1* ($p = 0.25$) and *COMT* ($p = 0.27$) genotypes were in Hardy–Weinberg equilibrium (Genepop version 4.0.10) (Rousset, 2008).

Statistical analyses

All statistical analyses were performed with SAS 9.1.3 software (SAS® Institute, Cary, NC). Fisher's exact tests were used to evaluate differences in frequencies and distributions. Demographic data and sleep-wake variables were analyzed with mixed-model analyses of variance (ANOVA) with the between-subject factors '*gender'* (male, female), '*DAT1 genotype'* (9R, 10R/10R), '*COMT genotype'* (Val/Val, Val/Met, Met/Met), and the covariate '*age'*, such as indicated in text and legends to Tables and Figures. For the circadian rest-activity profiles, the within-subject factors *time* (48 30-min bins across 24 h) was added. Differences in sleep duration between workdays and rest days were analyzed with an ANOVA with the between-subject factor '*COMT genotype'* (Val/Val, Val/Met, Met/Met), the within-subject factor *'type of day'* (workdays, rest days) and the covariate '*age'.* In text and tables, mean values ± standard deviations of outcome variables are reported. Contrasts were assessed with the LSMEANS statement of PROC MIXED. Only significant effects of factors and interactions are mentioned ($\alpha < 0.05$).

Results

Demographics of study sample

The data of 110 young healthy individuals were analyzed (Table 1). The study sample consisted of 65 men (59 %) and 45 women (41 %), with a mean age of 25.2 ± 3.8 years, 13.3 ± 2 years of education and a normal BMI (22.2 ± 2.3 kg/m^2). Chronotype (Munich Chronotype Questionnaire: 4.4 ± 1 [corrected midpoint of sleep]) and subjective sleepiness (Epworth Sleepiness Scale: 5.8 ± 2.8) ratings were also in the normal range. No significant differences among *DAT1* and *COMT* genotype groups with respect to sex ratio, age, years of education, chronotype, and daytime sleepiness were noted ($p_{all} > 0.05$; Fisher's exact tests). When split by either *DAT1* or *COMT* genotype, the distributions of genotype groups of the other gene did not differ ($p_{all} > 0.05$; Fisher's exact tests).

Study participants show robust circadian rest-activity profiles

Typical for our study sample consisting primarily of University students (77 %), NPCRA revealed high-amplitude, yet relatively irregular circadian rest-activity rhythms (Table 2). The latter was reflected in low IS scores due to large differences between workdays and rest days. The periods of lowest (L5) and highest (M10) activity began roughly 1 hour after actimetry-derived sleep onset and 2.7 hours after actimetry-derived sleep offset, respectively. The L5 initiated significantly earlier and revealed lower activity counts in women than in men (Table 2).

Time spent in bed (TIB) derived from the actimetry recordings correlated with self-reported TIB in the sleep-wake diaries ($R^2 = 0.51$, $p < 0.001$, $n = 109$; Pearson's product moment correlation). On average, the study participants slept 7.5 hours ± 36 min, with actigraphy-derived sleep onset at 00:36 ± 66 min and sleep offset at 08:06 ± 60 min (Table 3). Sleep onset and offset were affected by *'gender'* and *'type of day'*, such that these values were earlier in women than in men (Figure 1A) and earlier on workdays than on rest days (Figure 1 B). Sleep duration was not affected by *'gender'*. By contrast, sleep was shorter on workdays than on rest days. The average difference was 22 ± 51 min, yet highly variable among individuals (range: -89 to 173 min).

Table 1. Demographic characteristics of study participants.

Variable	10R/10R			9R carriers		
	Val/Val	Val/Val	Val/Met	Met/Met	Val/Met	Met/Met
N	21	27	27	15	23	10
Sex ratio (% Female)	43	44	21	40	48	40
Education (years)	13.0 ± 2.0	13.4 ± 1.8	13.2 ± 1.7	13.1 ± 2.8	13.3 ± 2.0	14.0 ± 2.4
Age (years)	25.2 ± 3.9	24.9 ± 3.9	26.5 ± 4.0	25.3 ± 3.7	25.0 ± 3.7	24.3 ± 3.8
Chronotype (MCTQ)	4.5 ± 1.0	4.2 ± 1.2	4.4±1.0	4.5 ± 1.1	4.4 ± 1.0	4.1 ± 1.1
Daytime sleepiness (ESS)	5.2 ± 2.5	5.3 ± 2.4	5.7 ± 3.2	6.5 ± 2.8	6.4 ± 3.4	6.5 ± 2.7
Body Mass Index (kg/m^2)	23.3 ± 2.4	21.4 ± 2.2	23.4 ± 2.3	22.2 ± 2.2	21.6 ± 2.4	22.0 ± 1.4

Variable	*'gender'*		*'DAT1 genotype'*		*'COMT genotype'*		*'DAT1' x 'COMT'*		*'age'*	
	F	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***
Education (years)	$F_{1,96}$=0.23	0.63	$F_{1,96}$=1.41	0.24	$F_{2,96}$=0.49	0.62	$F_{2,96}$=1.40	0.25	$F_{1,96}$=17.6	**< 0.0001**
Age (years)	$F_{1,100}$=0.00	0.98	$F_{1,100}$=0.29	0.59	$F_{2,100}$=0.15	0.86	$F_{2,100}$=0.56	0.57		
Chronotype (MCTQ)	$F_{1,99}$=0.43	0.52	$F_{1,99}$=0.00	0.96	$F_{2,99}$=0.31	0.31	$F_{2,99}$=0.12	0.88	$F_{1,99}$=0.98	0.33
Daytime sleepiness (ESS)	$F_{1,97}$=0.01	0.93	$F_{1,97}$=1.86	0.18	$F_{2,97}$=0.15	0.86	$F_{2,97}$=0.13	0.67	$F_{1,97}$=0.18	0.67
Body Mass Index (kg/m^2)	$F_{1,99}$=0.31	0.58	$F_{1,99}$=2.17	0.14	**$F_{2,99}$=3.36**	**0.04**	$F_{2,99}$=1.10	0.34	$F_{1,99}$=5.57	**0.02**

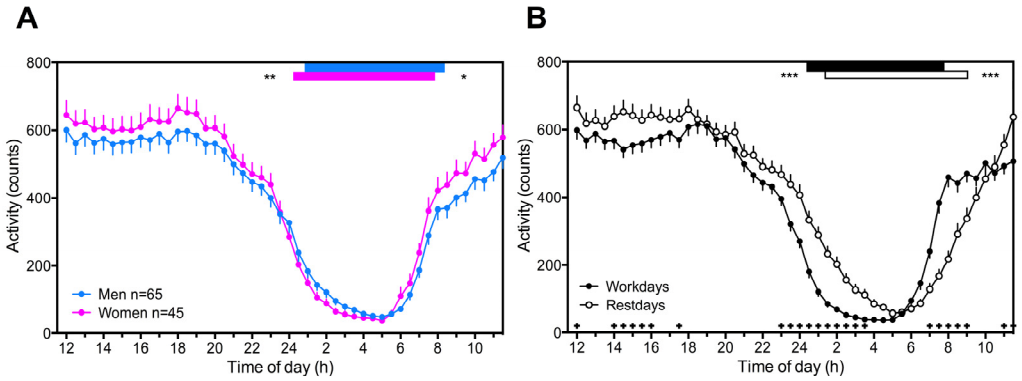
Values represent means ± STD. Information about education and daytime sleepiness was missing in 3 and 2 individuals, respectively. MCTQ values indicate the mid-sleep time on leisure days, corrected for sleep debt accumulated during the week, age and gender.
F- and p-values refer to mixed-model ANOVA with the factors '*gender*', '*DAT1 genotype*', '*COMT genotype*' and the covariate '*age*'.

Table 2. Non-parametric circadian rhythm analysis (NPCRA) reveals robust circadian rest-activity profiles.

Variable	Women n=45	Men n=65	*'gender'*		*'age'*	
			$F_{1,107}$	*p*	$F_{1,107}$	*p*
IS	0.40 ± 0.10	0.38 ± 0.12	*1.08*	*0.30*	*0.77*	*0.38*
IV	0.85 ±01.6	0.84 ± 0.17	*0.06*	*0.81*	*2.14*	*0.15*
Relative Amplitude	0.85 ± 0.08	0.81 ± 0.09	***4.33***	***0.04***	***5.33***	***0.02***
L5 onset	1.31 ± 0.92	1.77 ± 1.11	***5.23***	***0.02***	*3.37*	*0.07*
M10 onset	10.52 ± 1.52	10.93 ± 1.70	*0.05*	*0.82*	***4.87***	***0.02***
Amplitude	19428 ± 7393	18627 ± 6934	*0.36*	*0.55*	*3.72*	*0.06*
L5	1664 ± 1110	2161 ± 1427	***4.07***	***0.05***	***10.1***	***< 0.01***
M10	21092 ± 7686	20788 ± 7424	*0.05*	*0.82*	***5.67***	***0.02***

Values represent means ± STD in all participants split by gender. IS = inter-daily stability; IV = intra-daily variability; L5 = activity during the least 5 active hours; M10 = activity during the 10 most active hours.
F- and p-values refer to mixed-model ANOVA with the factor *'gender'* and the covariate *'age'.*

Figure 1. Rest-activity rhythms and sleep are affected by gender and type of day.



(**A**) 24-hour rest-activity profiles in men (n = 65) and women (n = 45). Means ± SEM are represented. Mixed-model ANOVA with the between-subject factor '*gender*', the between-subject factor '*time*' (30-min bins), and the covariate '*age*' (covariance structure: autoregressive type 1) revealed significant effects of '*time*' ($F_{48,5113}$ = 20.18, p < 0.0001) and '*age*' ($F_{1,228}$ = 10.43, p < 0.002). The actigraphy-derived sleep episode occurred roughly 30 minutes earlier in women than in men (horizontal bars; see Table 3 for mean values and statistics). (**B**) 24-hour rest-activity profiles on workdays (n = 19.5 ± 4.4 days per person) and rest days (n = 7.7 ± 2.1 days per person). Means ± SEM are represented. Mixed-model ANOVA with the within-subject factor '*type of day*' and the between-subject factor '*time*' (30-min bins) revealed significant effects of '*type of day*' ($F_{1,461}$ = 6.59, p = 0.01), '*time*' ($F_{48,4988}$ = 23.52, p < 0.0001), and '*time*' *x* '*type of day*' ($F_{48,5185}$ = 4.8, p < 0.0001). Crosses at the bottom of the panel indicate significant differences between workdays and rest days (p < 0.05; paired t-tests). The actigraphy-derived sleep episode occurred later and lasted longer on rest days than on workdays (horizontal bars; see Table 3 for mean values and statistics).

Table 3. Overview of actimetry-derived sleep variables.

Variable	Women n=45	Men n=65	'gender'		'age'		Workdays n=110	Restdays n=110	'type of day'	
			$F_{1,107}$	*p*	$F_{1,107}$	*p*			$F_{1,109}$	*p*
N (days/person)	28.0 ± 5.3	26.8 ± 6.4	*1.03*	*0.31*	*0.21*	*0.65*	19.5 ± 4.4	7.7 ± 2.1	***1257***	***<0.0001***
Time in bed (h)	7.9 ± 0.6	7.7 ± 0.7	*1.81*	*0.18*	*0.30*	*0.59*	7.7 ± 0.7	8.0 ± 0.9	***17.52***	***<0.0001***
Bedtime	24.1 ± 0.8	24.6 ± 1.3	***7.58***	***< 0.01***	***6.64***	***0.01***	24.1 ± 1.1	25.2 ± 1.4	***116.5***	***<0.0001***
Risetime	8.0 ± 0.8	8.4 ± 1.1	***5.01***	***0.03***	***6.71***	***0.01***	7.8 ± 1.0	9.2 ± 1.3	***142.0***	***<0.0001***
Sleep duration (h)	7.6 ± 0.6	7.4 ± 0.7	*2.21*	*0.14*	*0.26*	*0.62*	7.4 ± 0.7	7.8 ± 0.9	***19.60***	***<0.0001***
Sleep onset	24.3 ± 0.8	24.9 ± 1.3	***8.29***	***< 0.01***	***6.33***	***0.01***	24.3 ± 1.1	25.4 ± 1.4	***110.3***	***<0.0001***
Wake onset (sleep offset)	7.9 ± 0.8	8.3 ± 1.1	***5.44***	***0.02***	***6.65***	***0.01***	7.7 ± 1.0	9.1 ± 1.2	***142.0***	***<0.0001***
Midsleep	3.9 ± 0.6	4.6 ± 1.1	***6.83***	***0.01***	***7.07***	***0.01***	4.1 ± 1.0	5.1 ± 1.2	***41.4***	***<0.0001***
WASO (min)	37 ± 22	35 ± 15	*0.14*	*0.18*	*1.82*	*0.71*	35 ± 18	38 ± 19	***7.14***	***<0.01***
Sleep efficiency (%)	88.5 ± 4.4	88.3 ± 3.9	*0.07*	*0.80*	*1.80*	*0.18*	88.4 ± 4.2	88.5 ± 4.4	*0.40*	*0.53*
Sleep latency (min)	12 ± 4	14 ± 7	*3.06*	*0.08*	*0.11*	*0.74*	13 ± 6	12 ± 6	***8.49***	***< 0.01***
Mean activity	27 ± 14	26 ± 12	*0.12*	*0.73*	*1.43*	*0.23*	26 ± 13	27 ± 14	*3.32*	*0.07*
Total activity	7220 ± 3838	7010 ± 2599	*0.13*	*0.72*	*2.21*	*0.14*	6945 ± 3177	7471 ± 3504	***7.55***	***<0.01***
Δ sleep duration (min)	28 ± 55	18 ± 50	*0.93*	*0.34*	*1.02*	*0.31*	n.a.	n.a.		
Δ sleep time (min)	57 ± 50	64 ± 68	*0.43*	*0.51*	*3.09*	*0.08*	n.a.	n.a.		
Δ wake time (min)	85 ± 70	83 ± 76	*0.02*	*0.89*	*0.53*	*0.47*	n.a.	n.a.		

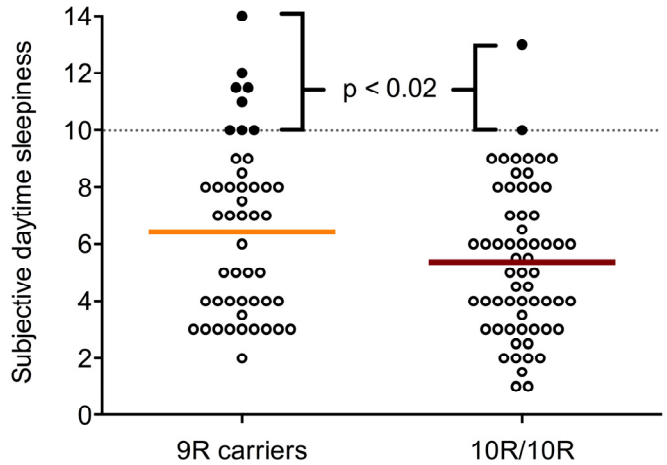
Values represent means ± STD, split by gender and type of day.
WASO: Wake after sleep onset. F- and p-values refer to mixed-model ANOVAs with the within-subject factor '*type of day'*, the between-subject factor '*gender'* and the covariate '*age'*.

The VNTR polymorphism of *DAT1* affects subjective sleepiness, and overall motor activity in men

Although mixed-model ANOVA revealed no significant main effect of *DAT1 genotype* on subjective sleepiness (Table 1), visual inspection of the individual data suggested that 9R carriers might be slightly sleepier than the 10R/10R homozygotes. Indeed, focusing on those individuals with clinically-defined elevated daytime sleepiness (Epworth sleepiness score over 10) (Johns, 2000) demonstrated that *DAT1* 9R (8/47 = 17.0%) carriers were more often present in this subgroup than 10R/10R homozygotes (2/61 = 3.3%) (Figure 2).

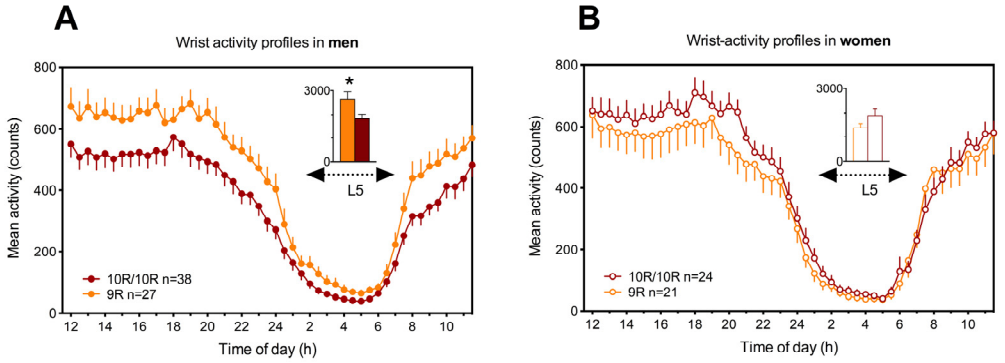
Whereas 24-hour motor activity profiles in the entire study sample did not differ significantly between men and women (Figure 1A), mixed-model ANOVA disclosed a sig-nificant *'DAT1 genotype'* x *'gender'* interaction ($F_{1,200}$ = 8.3, p < 0.005). Thus, male 9R carriers showed generally higher motor activity than 10R/10R homozygotes (Figure 3A), while this difference was absent in women (Figure 3B). The interaction between '*DAT1 genotype'* and *'gender'* was further corroborated when workdays ($F_{1,247}$ = 8.44, p = 0.004) and rest days ($F_{1,254}$ = 10.68, p < 0.002) were separately considered, and when the NPCRA outcome variable L5 ($F_{1,99}$ = 6.05, p < 0.02) was analyzed (insets in Figure 3A & 3B). In summary, the *DAT1* VNTR modulates circadian sleep-wake cycles in a gender-specific manner.

Figure 2. *DAT1* genotype affects subjective daytime sleepiness.



Data reflect individual scores on the Epworth Sleepiness Scale (n = 108). Black (ESS score ≥ 10) and white (ESS score < 10) circles represent individual data points. *DAT1* 9R carriers are more often present in the subgroup with elevated daytime sleepiness than 10R/10R homozygotes (p < 0.02; Fisher's exact test). Coloured horizontal bars represent the mean values in 9R (n = 47) and 10R/10R (n = 61) carriers.

Figure 3. *DAT1* polymorphism modulates circadian rest-activity profiles in a gender-dependent manner



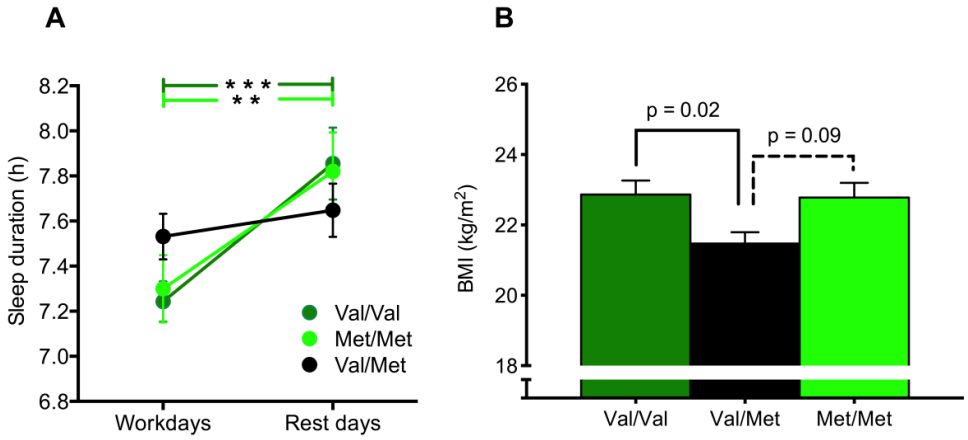
24-hour rest-activity profiles 9R and 10R/10R genotypes of *DAT1* in male (**A**) and female (**B**). Mixed-model ANOVA with the between-subject factors *'gender'*, *'DAT1 genotype'* and '*COMT genotype'*, the within-subject factor '*time'*, and the covariate '*age'* yielded significant effects of '*time'* ($F_{48,4732}$ = 17.37, p < 0.0001) and '*age'* ($F_{1,213}$ = 11.23, p = 0.001), and a significant '*DAT1'* x '*gender'* interaction ($F_{1,200}$ = 8.3, p < 0.005). Male 9R carriers had higher activity levels throughout the day when compared to 10R/10R homozygotes (p = 0.002; 2-tailed, unpaired t-test). Female study participants did not show this difference (p = 0.28). Activity during the least active five hours (L5; indicated by the horizontal dotted arrow), yielded a similar '*DAT1'* x '*gender'* interaction ($F_{1,99}$ = 6.05, p < 0.02). Orange (9R) and red (10R/10R) bars represent mean activity counts during L5 derived from the NPCRA analysis. The asterisk indicates the significant difference between the genotypes in men (p = 0.03; unpaired, 2-tailed t-test).

The Val158Met polymorphism of *COMT* affects the difference in actigraphy-derived sleep duration between workdays and rest days and BMI

The Val158Met polymorphism of *COMT* had no effect on subjective sleepiness and circadian rhythm variables. Nevertheless, mixed-model ANOVA with the between-subjects factors *DAT1 genotype*, *COMT genotype* and *gender*, and the covariate *age* revealed that *COMT* genotype affected the increase in time in bed ($F_{2,99}$ = 3.24, p = 0.04) and in sleep duration ($F_{2,99}$ = 3.34, p < 0.04) between workdays and rest days. The Val/Val and Met/Met homozygotes prolonged their sleep on rest days relative to workdays by 37 ± 58 and 31 ± 54 min. By contrast, this difference equalled only 7 ± 41 min in Val/Met individuals (Val/Met *vs.* Val/Val: p < 0.03; Val/Met *vs.* Met/Met: p < 0.08; 2-tailed, unpaired *t*-tests). A statistical analysis of sleep duration with *type of day* as within-subject factor confirmed the significant *COMT genotype* x *type of day* interaction ($F_{2,107}$ = 3.78, p = 0.03) (Figure 4A). Importantly, overall sleep duration was similar in the three genotypes, and did not significantly differ between genotypes, neither on workdays nor on rest days (p_{all} > 0.1; unpaired, 2-tailed t-tests).

A genotype-dependent association between sleep-wake timing across workdays and rest days and BMI was recently suggested for a *PER3* polymorphism (Lazar et al., 2012). We, therefore, examined whether the Val158Met polymorphism of *COMT* affects BMI in our healthy sample. Indeed, ANOVA confirmed a significant main effect of *COMT genotype* (Table 1). Specifically, BMI was lower in Val/Met heterozygotes when compared to Val/Val homozygotes ($p < 0.02$; unpaired, 2-tailed t-test) and tended to be lower in Val/Met heterozygotes when compared to Met/Met homozygotes ($p = 0.09$) (Figure 4B).

Figure 4. *COMT* genotype modulates sleep rebound from workdays to rest days and affects body-mass-index (BMI).



(**A**) Sleep duration on workdays and rest days in the three *COMT* genotypes. Mixed-model ANOVA with the between-subject factor '*COMT genotype',* the within subject factor '*type of day'* and the covariate '*age'* yielded a significant main effect of '*type of day*' ($F_{1,107} = 24.88$, $p < 0.001$) and '*type of day' x 'COMT genotype'* interaction ($F_{2,107} = 3.78$, $p = 0.03$). Thus, in contrast to Val/Val (dark green) and Met/Met (light green) homozygotes, Val/Met allele carriers (black) did not increase their sleep duration on rest days when compared to workdays. *** $p = 0.0001$, ** $p = 0.001$ (unpaired, 2-tailed t-tests). (**B**) Mixed-model ANOVA with the between-subject factors '*DAT1 genotype'*, *'COMT genotype'* and '*gender'*, and the covariate '*age',* yielded a significant effect of '*COMT genotype'* ($F_{2,99} = 3.36$, $p < 0.04$) and '*age'* ($F_{2,99} = 5.57$, $p = 0.02$). Val/Met allele carriers had lower BMI than Val/Val ($p = 0.02$) and Met/Met ($p < 0.10$) homozygotes (unpaired, 2-tailed t-tests).

Discussion

In an attempt to further specify the roles for dopamine in physiological sleep-wake regulation, we examined the effects of two functional polymorphisms in genes regulating cerebral dopamine levels on circadian rest-activity profiles and objective sleep estimates derived from actimetry. Consistent with our hypotheses, we found that a VNTR polymorphism of *DAT1* affects subjective sleepiness and modulates overall motor activity throughout activity and rest in men. Moreover, heterozygous Val/Met allele carriers of the Val158Met polymorphism of *COMT* showed consistently lower values than Val/Val and Met/Met homozygotes in their sleep rebound on rest days compared to workdays, as well as in their BMI.

The rs28363170 polymorphism of DAT1, individual subjective sleepiness and motor activity

We found that elevated daytime sleepiness was more prevalent in *DAT1* 9R allele carriers than in 10R/10R allele homozygotes. Excessive daytime sleepiness is a common symptom in patients with PD, a disease which is characterized by pathologically reduced dopamine levels in the striatum (Arnulf and Leu-Semenescu, 2009). Interestingly, a Taiwanese case-control study suggested that the homozygous 10R/10R genotype of *DAT1* confers protection against PD in men (Lin et al., 2003). Accumulating evidence in healthy adults demonstrates that the 9R allele is associated with higher DAT expression and lower dopamine availability than the 10R allele (Jacobsen et al., 2000; van Dyck et al., 2005; van de Giessen et al., 2009; Costa et al., 2011; Spencer et al., 2013). Our genetic data in healthy volunteers may, thus, suggest a possible pathophysiological link between the disabling sleepiness and the pronounced dopamine deficiency in PD patients.

This notion is further strengthened by the finding that male 9R carriers exhibited higher motor activity throughout days and nights compared to 10R/10R individuals. Elevated sleepiness and increased motor activity may be reminiscent of patients suffering from adult ADHD. This disorder more often afflicts males than females, and patients typically present with excessive daytime sleepiness, superficial sleep, elevated daytime and nighttime motor activity, and increased DAT expression when compared to controls (Dougherty et al., 1999; Dresel et al., 2000; Cheon et al., 2003; Spencer et al., 2005; Krause et al., 2006; Boonstra et al., 2007; Surman et al., 2009; Spencer et al., 2013). Thus, our finding of a behavioral ‘ADHD-like’ phenotype in healthy male 9R allele carriers who are presumably in a decreased dopaminergic

state when compared to 10R/10R homozygotes is in agreement with the hypo-arousal theory of ADHD (Swanson et al., 2007). This hypothesis posits that exaggerated physical activity and increased movements reflect a countermeasure to remain vigilant and alert in a condition of low dopaminergic tone.

The observed sexual dimorphism of the effects of *DAT1* polymorphism on motor activity may reflect the known impact of sex hormones and the menstrual cycle on the dopaminergic system (Dreher et al., 2007). Because the recording period in our study covered roughly an entire menstrual cycle, it is unlikely that cycle-related changes in DAT expression account for the different impact of *DAT1* genotype in men and women.

The rs4680 polymorphism of COMT, sleep-wake patterns and body weight regulation

With respect to the Val158Met polymorphism of *COMT*, we observed that Val/Met heterozygotes showed an attenuated increase in sleep duration on rest days when compared to workdays, and also presented with a lower BMI than homozygous genotypes. While no such association was present due to allelic variation in *DAT1*, Lazar et al. (2012) investigated the impact of a *PER3* polymorphism on sleep and health outcomes in 675 study participants. Similar to our results, they found that the group showing the highest BMI (*PER3*$^{5/5}$ genotype) also reported the largest difference in time in bed between workdays and rest days (Lázár et al., 2012). These observations are in accordance with a large-scale (n = 20'731) epidemiological study relying on a questionnaire, in which a positive association was found between BMI in overweight individuals (BMI ≥ 25) and the difference in midsleep time between free days and workdays (Roenneberg et al., 2012). While the number of participants in the present study is smaller than the previous samples, our work not only relied on questionnaires but also quantified sleep-wake patterns objectively over several weeks. It adds to the accumulating evidence that circadian rhythm irregularities and sleep-wake cycle disruptions contribute to individual differences in body-weight regulation, even in a population with normal weight and in the absence of shortened sleep duration.

Increasing evidence supports a role for dopamine in regulating food intake and modulating food reward via a meso-limbic circuitry (Schwartz et al., 2000; Wang et al., 2001). COMT metabolizes catechol-estrogens in peripheral tissue to 2-

methoxyestradiol, which influences fat regulation (Picó et al., 1998; Tworoger et al., 2004). Based on this background, a growing area of research has begun to explore potential associations between dopaminergic genes and obesity (Wang et al., 2001; Need et al., 2006). Indeed, an association between the Val158Met polymorphism of *COMT* and individual differences in food intake or BMI has been previously suggested (Tworoger et al., 2004; Galvão et al., 2012). Nevertheless, while the available studies revealed an inconsistent picture, comparison to our work is difficult because different research questions were addressed and in different populations with respect to age, gender, and health for example.

Distinct impact of DAT1 and COMT polymorphisms on rest-activity cycles

Our analyses disclosed no epistatic interactions between *DAT1* and *COMT* polymorphisms on rest-activity cycles in humans. The lack of such interactions may reflect the different regional distributions of DAT and COMT proteins in the brain. DAT is solely found in striatum (Ciliax et al., 1995), whereas COMT is mostly expressed in prefrontal cortex and to a lower extent in striatum (Meyer-Lindenberg et al., 2005; Tunbridge et al., 2006). Although the basal ganglia, including striatum, may be actively involved in regulating sleep-wake behavior (Lazarus et al., 2013), the main role of the dopaminergic neurons in the striatum is the regulation of motor activity. Indeed, *Dat* $^{-/-}$ mice display clear hyperactivity (Giros et al., 1996), and a *DAT* loss of function mutation in humans causes Infantile Parkinsonism-Dystonia with slow movements, rigidity and rest tremor (Kurian et al., 2009). Although our data were obtained in a rather small number of healthy individuals and await independent replication, they may be in line with the clinical manifestation of this fatal mutation.

Acknowledgments

We thank Carolin Reichert, Virginie Gabel, Micheline Maire, Darya Erkhova and Eva Junyent-Sallares for their help with recruitment of participants and data collection. We also thank Drs. Sarah Chellappa and Vivien Bromundt for general discussions about actimetry.

Declaration of Interest

The authors declare no conflicts of interest. This work was supported by the Swiss National Science Foundation (grant # 320030-135414), the University Research Priority Program ‘‘Integrative Human Physiology’’ (University of Zürich), and the Clinical Research Priority Program “Sleep & Health” (University of Zürich).

Chapter 3

Dopaminergic role in regulating neurophysiological markers of sleep homeostasis in humans

Sebastian C. Holst[1,3], Alessia Bersagliere[1], Valérie Bachmann[1,3], Wolfgang Berger[2,3,4], Peter Achermann[1,3,4], and Hans-Peter Landolt[1,3,4]

[1] Institute of Pharmacology and Toxicology, University of Zürich, Switzerland

[2] Institute of Medical Molecular Genetics, University of Zürich, Switzerland

[3] Zurich Center for Integrative Human Physiology, University of Zürich, Switzerland

[4] Neuroscience Center Zurich, University of Zürich & ETH Zürich, Switzerland

In revision: ***The Journal of Neuroscience***

Abstract

The roles for dopamine in regulating wakefulness and sleep are incompletely understood. Genetically modified mice lacking functional dopamine transporters (DAT) show prolonged wakefulness, shortened sleep, hypersensitivity to caffeine, and reduced-responsiveness to modafinil. Here we combined epidemiologic, pharmaco-genetic, and neurophysiologic methods to analyze the effects of the 3'-UTR variable-number-tandem-repeat polymorphism (SNP-ID: rs28363170) of the gene encoding DAT (*DAT1*, *SLC6A3*) on sleep-wake regulation in humans. Homozygous 10-repeat (10R/10R) allele carriers of this genetic variant presumably have reduced DAT protein expression than 9-repeat (9R) allele carriers. Consistent with the findings in mice, higher subjective caffeine sensitivity was associated with the 10R allele (n = 360) when compared to the 9R allele (n = 125). Moreover, caffeine and modafinil affected wakefulness-induced changes in functional bands (delta, sigma, beta) of rhythmic brain activity in wakefulness and sleep in *DAT1* genotype-dependent manner. Finally, the sleep deprivation-induced increase in established neurophysiological markers of sleep homeostasis, including slow wave sleep, EEG slow-wave activity (0.5-4.5 Hz), and number of individual low-frequency (0.5-2.0 Hz) oscillations in non-rapid-eye-movement sleep, was significantly larger in the 10R/10R genotype (n = 30) than in the 9R carrier genotype (n = 27). In conclusion, the data suggest that dopamine transporters contribute to sleep-wake regulation in humans.

Keywords:

DAT1; *SCL6A3*; caffeine; adenosine; EEG slow oscillation

Introduction

The dopamine transporter (DAT) is responsible for presynaptic re-uptake of dopamine from the synapse (Giros and Caron, 1993). Accumulating evidence suggests that dopamine and DAT contribute to the physiological regulation of wakefulness and sleep. *Drosophila* and mouse mutants lacking functional DAT exhibit prolonged wakefulness and shortened rest bouts or sleep (Giros et al., 1996; Wisor et al., 2001; Kume et al., 2005; Wu et al., 2008). Moreover, dopamine D_1 and D_2 receptor stimulation increases wakefulness in mice (Isaac and Berridge, 2003). In humans, the current evidence is less clear. While the dopamine precursor, L-dopa, and dopamine D_2/D_3 receptor agonists such as pramipexole may induce sleepiness and reduce wakefulness in healthy volunteers (Andreu et al., 1999; Micallef et al., 2009), loss of dopaminergic neurons in patients suffering from Parkinson's disease can cause different sleep-related symptoms, ranging from insomnia to increased daytime sleepiness (Park and Stacy, 2011).

The DAT protein is highly expressed in the striatum (Scatton et al., 1985; Lewis et al., 2001), where it constitutes the rate limiting mechanism of synaptic removal of dopamine (Jones et al., 1998; Benoit-Marand et al., 2000). Although behavioral and pharmacological findings in *Dat* knock-out mice need to be interpreted with caution, these animals appear to be hypersensitive to the stimulant effects of caffeine (Wisor et al., 2001). Although caffeine has no documented affinity for DAT, moderate doses of the stimulant may increase dopaminergic neurotransmission in the brain (Kaasinen et al., 2004). Thus, apart from blocking adenosine receptors (Fredholm et al., 1999), dopaminergic mechanisms may contribute importantly to wake-promotion by caffeine (Boutrel and Koob, 2004; Fisone et al., 2004; Andretic et al., 2008).

The concept of sleep homeostasis predicts that increased sleep need associated with prolonged wakefulness results in compensatory changes in sleep duration and intensity in recovery sleep (Borbély, 1982). The proportion of deep non-rapid-eye-movement (NREM) sleep, referred to as slow wave sleep (SWS), as well as EEG slow-wave activity (power within ~ 0.5-4.5 Hz) and number, amplitude and slope of individual slow (0.5-2.0 Hz) waves in NREM sleep after sleep deprivation are robust physiological markers of sleep homeostasis (Massimini et al., 2007; Bersagliere and Achermann, 2010; Achermann and Borbély, 2011). The neurochemical and molecular bases underlying this important regulatory mechanism of wakefulness and sleep are still poorly understood. In humans, pharmaco-genetic studies suggest that

caffeine interferes with sleep homeostasis by blocking adenosine A_{2A} receptors (Landolt et al., 2004; Rétey et al., 2006; 2007; Landolt, 2008; Bodenmann et al., 2012). These receptors are highly expressed in striatum and functionally interact with dopamine D_1 and D_2 receptors (Lovinger, 2010).

Wake-promoting agents such as cocaine, amphetamine, and also modafinil, increase dopaminergic neurotransmission by inducing the release of dopamine and inhibiting DAT (Kilty et al., 1991; Kahlig et al., 2004; Volkow et al., 2009a). Newertheless modafinil does not affect neurophysiological markers of sleep need in humans (Bodenmann et al., 2009b; Bodenmann and Landolt, 2010) nor does modafinil impact *Dat* knock-out mice (Wisor et al., 2001). From these studies, however, it cannot be concluded that dopamine plays no role in sleep homeostasis. Indeed, a recent study of our laboratory suggested that functional polymorphisms of the genes encoding COMT and DAT distinctly affect actigraphy-derived activity-rest patterns and sleep estimates in humans (Valomon et al., unpublished observations) (see Chapter 2).

Here we analyzed epidemiologic, pharmaco-genetic, and neurophysiologic data to examine the effects of the 3'-UTR variable-number-tandem-repeat polymorphism (SNP-ID: rs28363170) of the gene encoding DAT (*DAT1*, *SLC6A3*) on sleep-wake regulation in humans. This 40-bp sequence may occur in forms from 3 to 11 repeats, yet the presence of 9 or 10 repeats is most common (Vandenbergh et al., 1992). Accumulating evidence in young healthy adults indicates that 10-repeat (10R) allele homozygotes have 15-20 % reduced DAT availability in the striatum when compared to hetero- and homozygous 9-repeat (9R) allele carriers (Jacobsen et al., 2000; van Dyck et al., 2005; van de Giessen et al., 2009; Costa et al., 2011; Spencer et al., 2013). Based on the observations in *Dat* knock-out mice, we hypothesized that caffeine, modafinil, and sleep deprivation affect neurophysiologic markers of sleep homeostasis to a different extent in 10R/10R and 9R allele carriers of *DAT1*. Specifically, we expected that caffeine and sleep loss modify SWS, SWA, and individual EEG slow waves in recovery sleep stronger in 10R/10R homozygotes than in 9R allele carriers.

Materials and Methods

Study participants and genotyping

The study protocol was approved by the ethics committee of the Canton of Zürich for research on human subjects. Written informed consent was obtained from all participants prior to the experiments, as required according to the principles in the Declaration of Helsinki. All subjects received financial compensation for their participation.

A total of 504 healthy volunteers (374 males, 104 females) between 18 and 35 years of age were investigated. They were interested in participating in sleep studies and provided 3 ml fresh EDTA-blood (n = 472) (Wizard® Genomic DNA Purification Kit, Promega, Madison, WI) or saliva (n = 32) (NucleoSpin®Blood Kit, Marchery-Nagel AG, Oensingen, Switzerland) for isolation of genomic DNA. Their genotype of the rs28363170 polymorphism of *DAT1* was determined by allele-specific PCR on an MJ Research PTC-225 thermal cycler (MJ Research/Bio-Rad, Reno, NV) at an annealing temperature of 67°C. Forward primer, 5'-tgtggtgtagggaacggcctga-3' and reverse primer 5'-cttcctggaggtcacggctcaa-3' with HOT FIREPol® DNA Polymerase was used. The 430-480 bp PCR products were analysed by agarose gel electrophoresis. All analyses were replicated at least once for independent confirmation of results. In total, 258 individuals (69 females) were 10R/10R allele carriers, 204 (56 females) were heterozygous 9R/10R allele carriers, and 23 (4 females) were homozygous 9R/9R allele carriers. Nineteen carriers of rare *DAT1* alleles were excluded from the analyses. The answer to the question "Do you consider yourself as caffeine sensitive?" (yes / no) was compared among the *DAT1* genotypes.

To examine a possible impact of the *DAT1* polymorphism on sleep-wake regulation, sleep and sleep EEG recordings before and after sleep deprivation were analysed in a subset of 57 genotyped, right-handed subjects (46 men and 11 women) who previously completed sleep studies in our laboratory. Given the low number of 9R/9R homozygotes (n=3), homozygous (10R/10R) and heterozygous (9R/10R) 9R allele carriers (n = 27) were grouped together and compared to 10R/10R homozygotes (n = 30). These two genetic groups were previously found to differ in DAT expression (Jacobsen et al., 2000; van Dyck et al., 2005; van de Giessen et al., 2009). They were very similar in age, body-mass-index, habitual sleep

duration, daily caffeine and alcohol consumption, as well as in the distribution of distinct *COMT* and adenosine A_{2A} receptor (*ADORA2A*) genotypes (Table 1).

Table 1: Demographics

Variable	10R/10R	9R allele carriers	p-value
COMT genotype (rs4680; G/A)			
G/G (Val/Val)	13	11	
G/A (Val/Met)	8	7	
A/A (Met/Met)	9	9	
ADORA2A genotype (rs575876; T/C)			
T/T	8	4	
C/T	16	11	
C/C	6	12	
Sex ratio (% females)	25.0	22.7	
Age (years)	24.6 ± 0.6	24.7 ± 0.6	0.89
Body-mass-index (kg/m^2)	22.8 ± 0.4	22.0 ± 0.3	0.09
Habitual sleep duration (hours)	7.3 ± 0.2	7.4 ± 0.2	0.79
Caffeine consumption (mg/day)	139.0 ± 26.0	103.6 ± 22.5	0.31
Alcohol consumption (drinks/week)	3.2 ± 0.6	2.8 ± 0.4	0.60
Trait Anxiety (STAI)	39.3 ± 1.5	36.9 ± 1.5	0.26
Daytime Sleepiness (ESS)	7.2 ± 0.5	7.0 ± 0.5	0.81

NCBI symbols indicate genes encoding dopamine transporter (*DAT1*), catechol-O-methyltransferase (*COMT*), and adenosine A2A receptor (*ADORA2A*). The distributions of *COMT* ($p = 1.0$) and *ADORA2A* ($p = 0.15$) genotypes, and the sex ratio in 10R/10R and 9R allele carriers of *DAT1* did not differ between the two groups (Fisher's Exact Tests). Values represent means ± SEM. *P*-values refer to 2-tailed Student's *t*-tests. All subjective estimates were based on validated self-report questionnaires. STAI: State-trait anxiety inventory (Spielberger et al., 1970) ESS: Epworth Sleepiness Scale (Johns, 1991).

All volunteers reported to be good sleepers, adhering to regular bedtimes, to be in good physical health, and to have a medical history free of neurological and psychiatric disorders. Two months prior to enrollment, subjects stated not to have consumed any medication or illicit drugs, not to have passed through time zones and to consume moderate amounts of caffeine and alcohol. To check for potential undiagnosed sleep disorders or low sleep efficiency (< 85%), they underwent a screening night in the sleep laboratory prior to study inclusion. Two weeks prior to study initiation, participants were instructed to wear a wrist activity monitor on their non-dominant arm, to fill out a sleep-wake diary, and to completely refrain from intake of caffeine. The final 3 days before the start of the experiment, they were required to strictly maintain regular bedtimes (8-hours sleep) and to abstain from alcohol.

Sleep deprivation protocol

The sleep deprivation study involved either one (n = 19 [12 females, 7 male]) or two (n = 38, all males) experimental blocks. Three participants completing only one block were moderate smokers (< 10 cigarettes per day). Each experimental block consisted of three nocturnal sleep recordings, all starting either at 23:00 or 00:00, divided into 8-hour adaptation and baseline nights, 40 hours of constantly supervised prolonged wakefulness, and concluded by a 10-hour recovery night.

Subjects completing two experimental blocks were administered after 11 (at 19:00) and 23 hours (at 07:00) of prolonged wakefulness two times 200 mg of caffeine and placebo (n = 16), or two times 100 mg modafinil and placebo (n = 22), in randomized, double blind, cross-over fashion.

All-night polysomnography

Sleep was polysomnographically quantified during all experimental nights. The EEG, electrooculogram (EOG), submental electromyogram (EMG), and electrocardiogram (ECG) were recorded using polygraphic amplifiers (PSA24, Braintronics Inc., Almere, The Netherlands (Bachmann et al., 2012a); or Artisan, Micromed, Mogliano Veneto, Italy (Rétey et al., 2006)) as follows: For 16 subjects recorded via PSA24 amplifiers, analog EEG data were band-pass filtered (-3 dB at 0.16 Hz; -3 dB at 102Hz) and sampled at 512 Hz, then digitally low pass filtered (-3 dB at 49 Hz) and stored with a resolution of 128 Hz. For 41 subjects recorded via Artisan amplifiers, analog EEG data were band-pass filtered (-3 dB at 0.15 Hz; -3 dB at 67.2 Hz), sampled and stored with a resolution of 256 Hz. EEG data of derivation C3A2 were analyzed.

Sleep stages were visually scored in 20-s epochs according to standard criteria (Rechtschaffen and Kales, 1968). Four-s EEG spectra (fast Fourier transform routine, Hanning window, 0.25-Hz resolution) were calculated using MATLAB (MathWorks Inc., Natick, MA), averaged over 5 consecutive 4-s epochs, and matched with scored sleep stages. Arousal- and movement-related artifacts were semi-automatically identified and excluded. All-night power spectra between 0-20 Hz represent the average of all artifact-free 20-s values in NREM sleep (stages 1-4) and REM sleep. In the recovery nights, data analysis was restricted to the first 8 hours of the 10-h sleep recordings. All-night and episodic EEG power values in NREM sleep

were standardized in each individual to the corresponding all-night power of the baseline night.

To analyse the effects of caffeine, modafinil, *DAT1* genotype, and sleep deprivation on individual EEG slow waves, an algorithm previously described by (Bersagliere and Achermann, 2010) was applied. Data sampled with 256 Hz were down sampled to 128 Hz using the MATLAB function *decimate* (MathWorks Inc., Natick, MA). To ensure reliable wave detection, the EEG (0.5-2.0 Hz) was band-pass filtered (third-order Chebyshev type II high-pass filter; –3 dB at 0.4 Hz; sixth-order Chebyshev type II low-pass filter, –3 dB at 2.3 Hz). Filters were applied both in forward and reverse directions, to prevent phase distortion. Individual positive and negative half-waves with an amplitude of ≥ 37.5 μV (75 μV peak-to-peak) (Rechtschaffen and Kales, 1968) were defined as positive or negative deflections between consecutive zero-line crossings. Waves were analysed in steps of 0.1 Hz. The amplitude was defined as the local maximum or minimum between two consecutive zero line crossings, whereas the slope was defined as the amplitude of the peak or the trough, divided by the time from the previous zero-line crossing (initial mean slope). The number of waves included all detected waves that fulfilled the ≥ 37.5 μV-amplitude criterion.

Waking EEG recordings

At 3-hour intervals during the 40 hours prolonged wakefulness, standardized waking EEG recordings were performed. At least 1 hour before each recording, subjects had to stay in the laboratory (constant temperature: 19-21 ^{o}C; light intensity: < 150 lux). Fifteen minutes before each recording, they calmly stayed on their own in their bedrooms. The study participants were instructed to comfortably relax in a chair, and to place their chin on an individually adjusted chinrest. An initial 3-min recording period with eyes closed was followed by a 5-min period with eyes open, while subjects fixated a black dot attached to the wall. Artifacts in all derivations were visually identified and excluded. Power spectra (C3A2 derivation) of artifact-free, 2-s epochs (50 % overlap, Hanning window, frequency resolution 0.5 Hz) were computed and analyzed between 0-25 Hz. To minimize inter-individual differences in absolute power and to compensate for circadian effects, spectra were standardized in each individual to the mean of the first four sessions during extended waking.

Statistical analyses

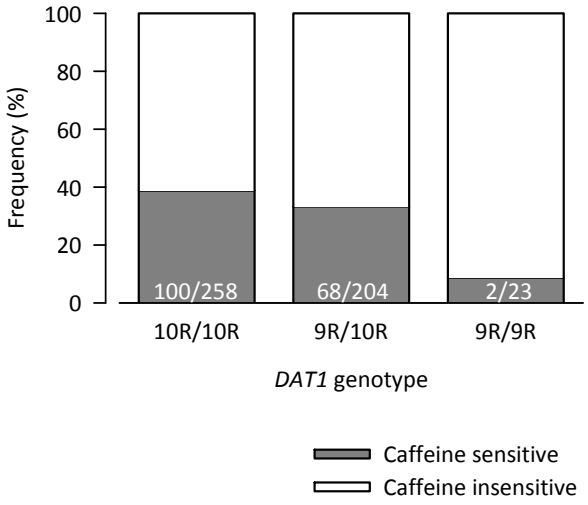
All statistical analyses were performed with SAS 9.1.3 software (SAS Institute, Cary, NC). In 10R/10R and 9R allele carriers of *DAT1*, self-rated caffeine sensitivity, the effects of caffeine and modafinil on waking EEG, sleep architecture, EEG spectra, and individual EEG slow half-waves, as well as the sleep deprivation-induced rebound in these variables were analyzed. Two- and 3-way mixed-model analyses of variance (ANOVA) for repeated measure (PROC MIXED) with the between-subjects factor *'genotype'* (10R/10R *vs.* 9R allele carriers) and the within-subjects factors *'treatment'* (caffeine / modafinil *vs.* placebo), *'NREM sleep episode'* (1-4), *'condition'* (baseline *vs.* sleep deprivation / recovery), and *'frequency bin'* (0.5-, 0.25-, or 0.1-Hz resolution) were performed. The significance level was set at $\alpha < 0.05$. Only significant effects and interactions are mentioned, unless otherwise specified. Post-hoc analyses were only performed when the respective main effect and/or interaction of the ANOVA were significant.

Results

Highest self-rated caffeine sensitivity in 10R/10R allele carriers of *DAT1*

To test the hypothesis that genetically determined differences in DAT availability could affect sensitivity to caffeine, self-rated caffeine sensitivity was compared 10R/10R, 9R/10R, and 9R/9R allele carriers of *DAT1*. The proportion of sensitive individuals differed among the genotypes ($p < 0.008$, Fisher's exact test). It equaled 38.8 % in 10R/10R allele carriers, 33.3 % in 9R/10R allele carriers, and 8.7 % in 9R/9R allele carriers (Figure 1). The difference was most pronounced when homozygous 10R and 9R carriers were compared.

Figure 1. *DAT1* genotype affects self-rated caffeine sensitivity.

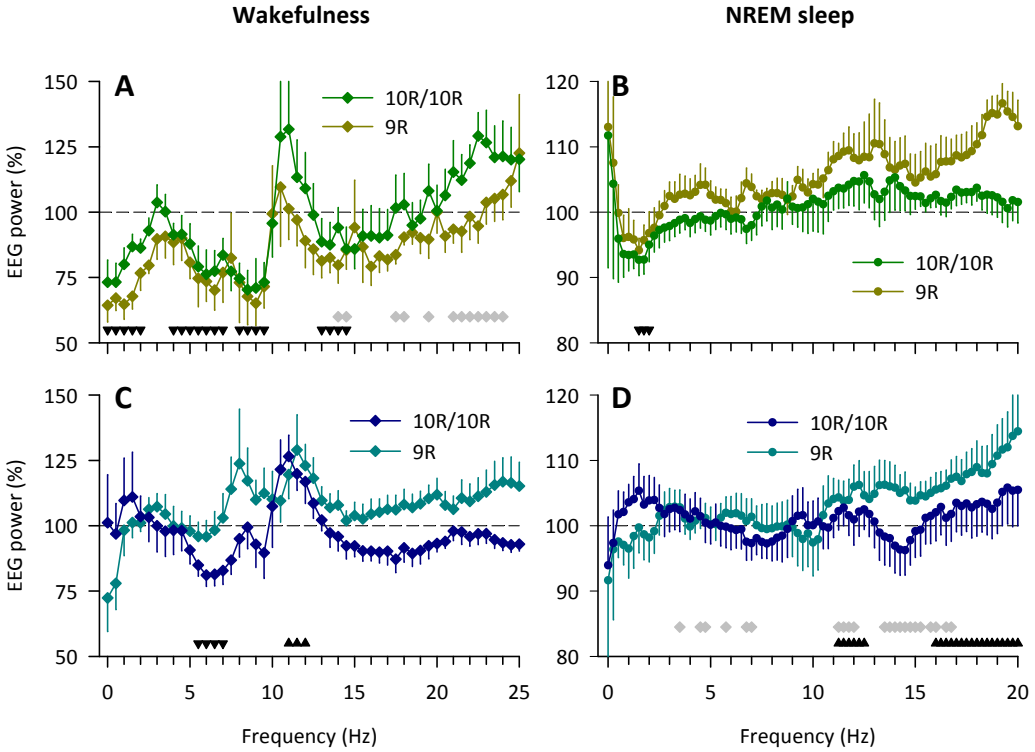


Five-hundred and four healthy adults rated themselves as either being caffeine sensitive or caffeine insensitive. Numerators and denominators at the bottom of the bar represent the numbers of caffeine sensitive individuals and the total numbers of subjects in each genotype.

DAT1 genotype modulates effects of caffeine on low-frequency brain oscillations in wakefulness and sleep

To investigate a possible role for DAT in sleep-wake regulation, the combined effects of sleep deprivation and caffeine and modafinil were examined in a data set of 57 healthy adult 10R/10R and 9R allele carriers (Table 1). Sleep architecture in baseline, and the behavioral effects of prolonged wakefulness (i.e., increased sleepiness and impaired performance on the psychomotor vigilance task; data not shown) were highly similar in the two genotypes.

Figure 2. *DAT1* genotype dependent modulation of caffeine and modafinil.



The DAT1 genotype modulates the effects of caffeine (10R/10R: n = 7 [green symbols]; 9R allele carriers: n = 9 [olive symbols]) and modafinil (10R/10R: n = 11 [blue symbols]; 9R allele carriers: n = 11 [dark cyan symbols]) intake during sleep deprivation on EEG activity in wakefulness (eyes open) and NREM sleep. (**A** & **B**) Effects of caffeine. (**C** & **D**) Effects of modafinil. *Left panels:* Relative power values in the waking EEG recording sessions at 08:00, 11:00, 14:00, and 17:00 on day 2 of prolonged wakefulness after caffeine (2 x 200 mg) and modafinil (2 x 100 mg) were expressed as a percentage of the corresponding values after placebo (horizontal dashed lines at 100 %). Caffeine reduced power in many bins below 14.5 Hz, and increased power in high-beta (21-24 Hz) frequencies (in 10R/10R carriers only). Modafinil reduced theta (5.5-7 Hz) activity and increased alpha (11-12 Hz) activity. *Right panels:* Standardized all-night power values in NREM sleep (stages 1-4) after caffeine (2 x 200 mg) and modafinil (2 x 100 mg) intake were expressed as a percentage of the corresponding values after placebo (horizontal dashed lines at 100 %). Caffeine reduced 1.5-2 Hz activity. Modfinil increased power in many bins in the alpha/beta range (11.25-20 Hz), primarily in 9R carriers of *DAT1*. Means ± SEM of consecutive 0.5-Hz (wakefulness) or 0.25-Hz (NREM sleep) frequency bins are plotted. Grey diamonds (caffeine: $F_{1,14} \geq 4.25$; modafinil: $F_{1,20} \geq 4.49$; $p_{all} < 0.05$) indicate a significant *'genotype'* x *'treatment'* interaction of a 2-way mixed-model ANOVA with the between subjects factor *'genotype'* and the within-subjects factor *'treatment'* (caffeine or modafinil). Up- and downward black triangles (caffeine: $F_{1,14} \geq 4.61$; modafinil: $F_{1,20} \geq 5.13$; $p_{all} < 0.05$) indicate a significant main effect of *'treatment'*. Note the different scales of y- and x-axes in wakefulness and NREM sleep.

Caffeine intake during sleep deprivation affected EEG low-frequency activity in wakefulness and sleep. The stimulant reduced waking EEG activity in delta (< 2 Hz), theta (4 - 7 Hz), alpha (8 - 9.5 Hz) and low-beta (13 - 14.5 Hz) frequencies (Figure 2A). Moreover, consistent with increased subjective sensitivity to its stimulant actions, the xanthine enhanced beta (21 - 24 Hz) activity in 10R/10R carriers

(caffeine: 151.6 ± 9.5 %, placebo: 109.3 ± 9.7 %, t = -2.40, $p < 0.05$; paired, 2-tailed t-test), yet not in 9R carriers (caffeine: 103.9 ± 11.4 %, placebo: 127.2 ± 4.5 %, t = 1.51, $p > 0.18$). During recovery sleep, caffeine consistently reduced EEG low-delta (1.5 - 2.0 Hz) activity in NREM sleep (Figure 2B). Close inspection of the data indicated that this effect of caffeine may differ between 10R/10R and 9R allele carriers of *DAT1*. To further examine this notion, individual sleep slow waves were analyzed.

DAT1 genotype modulates the effects of caffeine on individual slow waves in NREM sleep

Sleep deprivation markedly increased number, amplitude, and slope of individual, positive and negative slow half-waves in recovery sleep when compared to baseline sleep (three-way mixed model ANOVA, 'condition': $F_{1,55} > 93.7$, $p_{all} < 0.0001$). Intake of caffeine during prolonged wakefulness reduced all these features of negative half-waves, as well as number and amplitude of positive half-waves in 10R/10R carriers of *DAT1* (Figure 3). By contrast, the stimulant did not affect the characteristics of positive and negative slow half-waves in 9R allele carriers.

Modafinil does not affect low-frequency brain oscillations in wakefulness and sleep

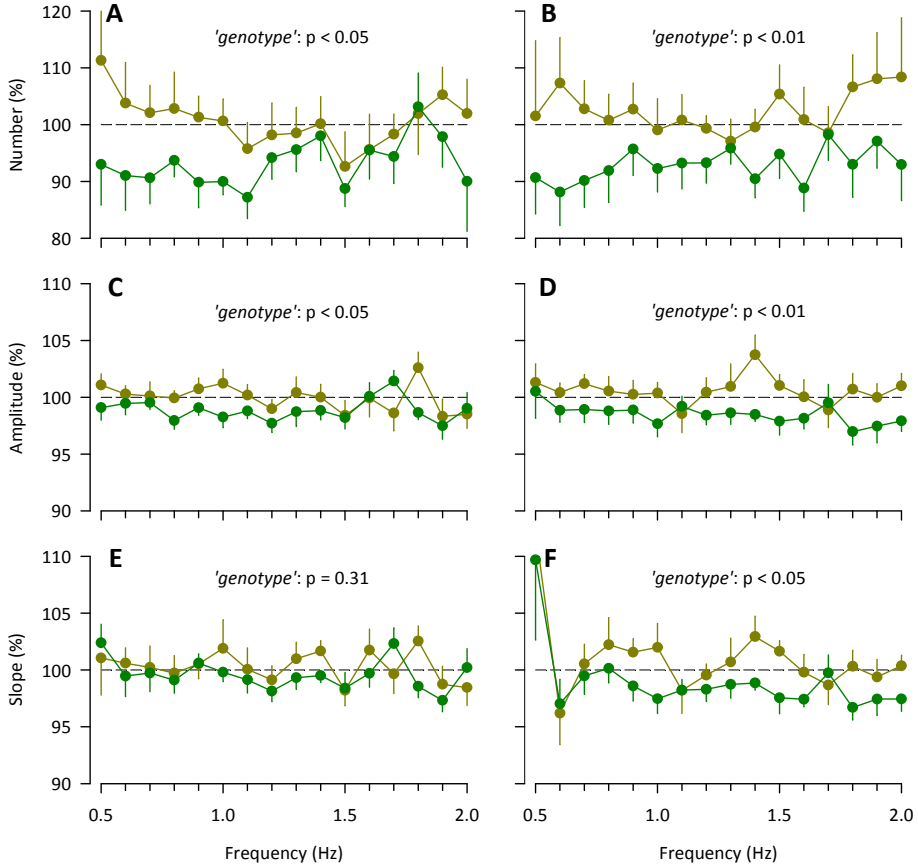
Intake of modafinil during prolonged wakefulness reduced waking EEG activity in the 5.5 - 7 Hz range and elevated activity in the 11 - 12 Hz band when compared to placebo (Figure 2C). In the recovery night, modafinil did not affect low-frequency EEG activity in NREM sleep (Figure 2D), or the number, amplitude and slope of EEG slow half-waves (data not shown). Nevertheless, the stimulant increased power in alpha (11.25 - 12.5 Hz) and beta (13.5 - 20 Hz) frequencies in NREM sleep, primarily in 9R carriers of *DAT1* (Figure 2D).

DAT1 genotype modulates the rebound in slow wave sleep and EEG slow-wave activity after sleep deprivation

Because the effects of caffeine in NREM sleep reflected *DAT1* genotype-dependent changes in EEG slow oscillations, the repercussions of prolonged wakefulness on these neurophysiologic markers of sleep homeostasis were examined in 10R/10R and 9R allele carriers of *DAT1*. In both genotypes, sleep deprivation increased sleep efficiency and the duration of NREM sleep, and shortened sleep latency, sleep stages 1 and 2, REM sleep, and wakefulness after sleep onset in the recovery night when compared to the baseline night (data not

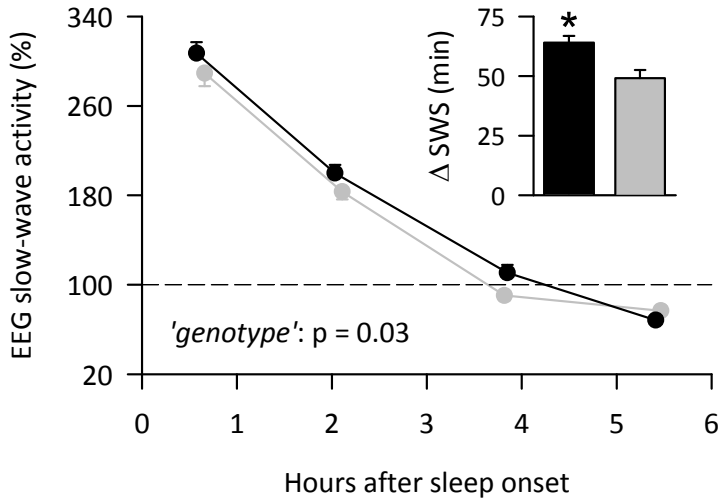
shown). Nevertheless, the rebound in deep SWS was roughly 15 min longer in 10R/10R than in 9R allele carriers (Figure 4, inset). This difference was accompanied by a slightly more pronounced reduction in the 10R/10R genotype in stage 2 sleep (DAT 9R: -9.8 min, DAT 10R/10R: -21.6 min, two-way mixed model ANOVA, 'genotype x condition': $F_{1,55} = 3.0$, $p = 0.09$).

Figure 3. *DAT1* genotype modulates individual slow half-waves in a caffeine dependent manner.



DAT1 genotype modulates the effects of caffeine intake during sleep deprivation on positive (left) and negative (right) EEG slow half-waves in NREM sleep. Number **(A & B),** amplitude **(C & D),** and slope **(E & F)** in 10R/10R (green lines, n = 9) and 9R allele carriers (olive lines, n = 7) genotypes of *DAT1* are illustrated. Absolute data were normalized to the corresponding recovery night after intake of placebo (horizontal dashed line at 100 %) and used for analyses. P-values refer to the factor *'genotype'* of a 2-way, mixed-model ANOVA with the between-subject factor *'genotype'* and the within-subject factor *'frequency bin'*. Error bars denote ± SEM.

Figure 4. SWS and EEG-SWA is affected by the *DAT1* genotype.

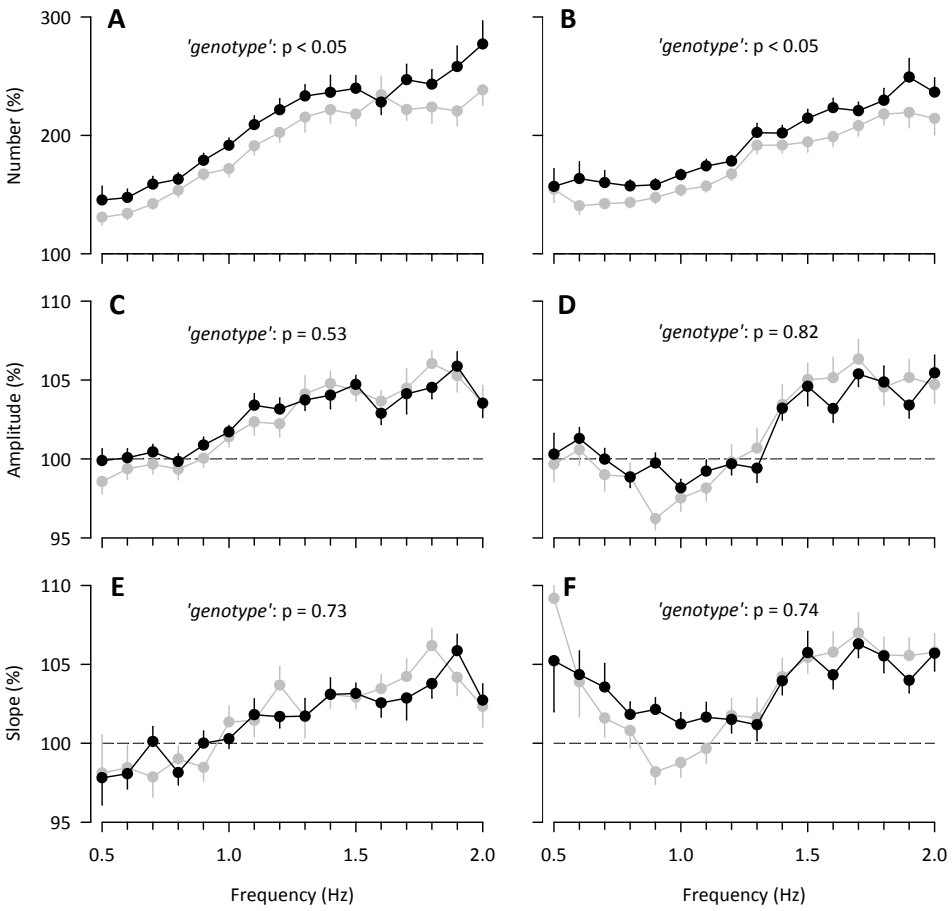


DAT1 genotype modulates the rebound in slow wave sleep (SWS) and EEG slow-wave activity (SWA, 0.5-4.5 Hz) in the recovery night after sleep deprivation. *Inset:* Data reflect the mean absolute all-night increase in SWS after sleep deprivation when compared to baseline, in 10R/10R (black bar, n = 30) and 9R allele carriers (grey bar, n = 27) genotypes of *DAT1*. Two-way mixed-model ANOVA: *'condition'*: $F_{1,55}$ = 644.1, p < 0.0001; *'genotype'*: ns; *'condition' × 'genotype'*: $F_{1,55}$ = 10.8, p < 0.002. Asterisk: p < 0.002 (2-tailed *t*-test). *Main figure*: Mean relative SWA, expressed as a percentage of the all-night value in baseline (horizontal dashed line at 100 %). Values in 10R/10R (black dots, n = 30) and 9R allele carriers (grey dots, n = 27) across the first 4 NREM sleep episodes were plotted at the mean episode midpoints relative to sleep onset. Two-way mixed-model ANOVA on relative values: *'genotype'*: $F_{1,55}$ = 4.8, p = 0.03; *'NREM sleep episode'*: $F_{3,165}$ = 372.7, p < 0.0001; *'genotype' × 'NREM episode'*: ns). Error bars indicate ± SEM.

In baseline and recovery nights, EEG SWA (0.5-4.5 Hz range) decayed exponentially across consecutive NREM sleep episodes, reflecting the dissipation of homeostatically regulated NREM sleep pressure. The time constants of the decay (τ_d) quantified by the method proposed by (Borbély, 1982; Rusterholz et al., 2010) were similar in both nights and genotypes (data not shown). Interestingly, however, the rebound in SWA in the recovery night compared to the baseline night was larger in 10R/10R than in 9R allele carriers of *DAT1* (Figure 4).

The analyses of individual EEG slow waves revealed that this difference reflected a more pronounced increase in 10R/10R than in 9R allele genotypes in the number of positive and negative half-waves after sleep deprivation (Figure 5). By contrast, the changes in amplitudes and slopes of individual slow-waves in NREM sleep did not differ between the genotypes.

Figure 5. The *DAT1* genotype modulate effects of sleep deprivation on individual slow half-waves.



DAT1 genotype modulates the effects of sleep deprivation on positive (left) and negative (right) EEG slow half-waves in NREM sleep. Number (**A** & **B**), amplitude (**C** & **D**), and slope (**E** & **F**) in 10R/10R (black lines, n = 30) and 9R allele carriers (grey lines, n = 27) genotypes of *DAT1* are illustrated. Normalized data relative to the corresponding all-night baseline values (= 100 %) were analyzed. P-values refer to the factor *'genotype'* of a 2-way, mixed-model ANOVA with the between-subject factor *'genotype'* and the within-subject factor *'frequency bin'*. Error bars indicate ± SEM.

Discussion

The dopamine transporter (DAT) is responsible for re-uptake of dopamine into presynaptic neurons (Giros and Caron, 1993) and regulates synaptic dopamine availability primarily in the striatum (Jones et al., 1998; Benoit-Marand et al., 2000; Jaber et al., 2004). The 3'-VNTR polymorphism of the *DAT1* gene examined here has been linked to reduced DAT protein expression in homozygous 10R/10R allele carriers when compared to 9R allele carriers (Heinz et al., 2000; Jacobsen et al., 2000; Fuke et al., 2001; van Dyck et al., 2005; VanNess et al., 2005; van de Giessen et al., 2009). We investigated the impact of this polymorphism as an "experiment of nature", to examine the effects of genetically altered striatal dopaminergic neurotransmission on wake-sleep regulation in healthy humans. Based on preclinical studies in DAT mutant animals (Giros et al., 1996; Wisor et al., 2001; Kume et al., 2005; Wu et al., 2008), as well as molecular brain imaging data in humans (Volkow et al., 2012), we hypothesized that genetically-determined differences in striatal dopaminergic neurotransmission have an impact on sleep-wake regulation. Our data revealed not only pharmaco-genetic evidence for this hypothesis, but also demonstrated that healthy carriers of the 10R/10R genotype show increased rebound in SWS, SWA, and number of slow EEG waves after sleep deprivation when compared to 9R allele carriers. These findings suggest an increased homeostatic response to sleep deprivation in 10R/10R carriers compared to 9R carriers of *DAT1*.

The mode of action of the stimulants caffeine and modafinil has been suggested to rely directly or indirectly on the dopaminergic system, and to depend on DAT expression (Wisor et al., 2001; Boutrel and Koob, 2004; Fisone et al., 2004; Andretic et al., 2008). In view of caffeine, our study revealed that *DAT1* genotype modulates subjective caffeine sensitivity, such that 10R/10R homozygotes reported higher sensitivity than 9R allele carriers. A more pronounced response to caffeine in the 10R/10R genotype was confirmed objectively in the elevation of 21 - 24 Hz activity in wakefulness (Figure 2A). These data are consistent with findings in genetically modified mice (Wisor et al., 2001). They suggest enhanced caffeine sensitivity with reduced expression of DAT, such as in 10R/10R allele carriers of *DAT1* and in *Dat* knock-out mice. This observation indicates that adenosine-dopaminergic interactions are important in determining the stimulant actions of the adenosine receptor antagonist, caffeine.

Our study showed for the first time in humans that this conclusion may also apply to the effects of caffeine on neurophysiologic markers of sleep homeostasis. Intake of the stimulant during prolonged wakefulness reduced EEG low-frequency activity in the delta (wakefulness: 0 - 2 Hz; NREM sleep: 1.5 - 2 Hz) and theta (wakefulness: 4 - 9.5 Hz) ranges in vigilance state-specific manner (Figure 2A & 2B). By contrast, administration of modafinil, which reduces DAT-mediated re-uptake of dopamine in animals (Mignot et al., 1994) and humans (Volkow et al., 2009a), decreased theta power in a narrow band (5.5 - 7 Hz) in waking, yet had no effect on delta activity in NREM sleep (Figure 2C & 2D). It was previously concluded that pharmacological blockade of adenosine receptors (by caffeine) and enhancement of dopaminergic neurotransmission (by modafinil) during sleep deprivation differentially affect distinct markers of sleep homeostasis (Bodenmann et al., 2009b; Bodenmann and Landolt, 2010). The present refined EEG analyses revealed that caffeine reduced number, amplitude, and slope of individual slow waves in a *DAT1* genotype-dependent manner. This finding suggests that the interference of caffeine with neurophysiologic markers of sleep homeostasis not only relies on adenosinergic mechanisms but also involves dopaminergic processes.

The rebound after prolonged wakefulness in SWS, SWA, and the number of individual slow half-waves in NREM sleep was significantly larger in 10R/10R allele homozygotes than in 9R allele carriers. A recent study based on a lower number of participants (n = 27 men) and lacking quantitative EEG analyses and sleep deprivation, reported no effect of the *DAT1* VNTR on sleep architecture (Guindalini et al., 2010). By contrast, our findings derived from fine-grained EEG analyses demonstrate that DAT contributes to sleep-wake regulation. Individual low-frequency EEG oscillations in the 0.5 - 2 Hz range in NREM sleep are homeostatically regulated (Bersagliere and Achermann, 2010) and thought to reflect at the cellular level the degree of synchrony in the occurrence of up- and down-states in the membrane potential of cortical neurons (Mukovski et al., 2007; Murphy et al., 2009; Vyazovskiy et al., 2009; Bersagliere and Achermann, 2010). Sleep loss increased in genotype-dependent manner the number of slow waves, but not their amplitude and slope (Figure 5). Together with the data presented in Figure 4, this observation suggests the conclusion that *DAT1* modulates the duration of the sleep deprivation-induced rebound in SWS, rather than the absolute level of SWA that reflects the synchrony of up- and down-states in cortical networks.

When relating our data to clinical and preclinical findings, a complex role for striatal dopamine in sleep-wake regulation emerges. More specifically, many patients with Parkinson's disease suffer from severely disturbed sleep and disabling daytime sleepiness (Park and Stacy, 2011). These patients have pathologically impaired dopaminergic neurotransmission in the striatum. We recently observed that elevated daytime sleepiness and increased motor activity are more prevalent in healthy 9R allele carriers of *DAT1* when compared to 10R/10R homozygotes (Valomon et al., unpublished observations) (see Chapter 2). Together with the stronger response to caffeine and the larger rebound in neurophysiologic markers of sleep homeostasis after sleep deprivation presented in this study, the findings are consistent with reduced DAT expression and increased dopaminergic signaling in 10R/10R allele carriers. Furthermore, pronounced hyperactivity, caffeine hypersensitivity, and reduced sleep duration are typically observed in *Dat* knock-out animals (Giros et al., 1996; Wisor et al., 2001; Kume et al., 2005; Wu et al., 2008). Nevertheless, because constitutive knock-out of *Dat* produces drastically increased dopaminergic tone and may cause pronounced behavioral changes, the relevance of this genetic intervention for the interpretation of the subtle effects of genetic variation of *DAT1* in healthy volunteers needs to be judged with caution.

In conclusion, our study provides novel physiological evidence for a functional effect of the common VNTR polymorphism of *DAT1*. Moreover, because the DAT protein is primarily expressed in the striatum, it suggests a specific role for striatal dopaminergic neurotransmission in sleep-wake regulation.

Notes

Supplemental material for this article is available in Appendix 1. The supporting supplemental material (one table and two figures) includes more detailed information on *DAT1* genotype dependent sleep architecture, analysis of the individual positive and negative slow half-waves for absolute data and for the estimates of SWA decay time constants (τ_d). This material has not been peer reviewed

.

Acknowledgments

This work was supported by the Swiss National Science Foundation (grant # 320030-135414), the University Research Priority Program ‘‘Integrative Human Physiology’’ (University of Zürich), and the Clinical Research Priority Program “Sleep & Health” (University of Zürich). We thank Dr. S Bodenmann and Dr. J. Rétey for

data collection and sleep stage scoring, and Dr. T. Rusterholz, Ms. A. Valomon and Dr. R. Dürr for their help with data analyses.

Chapter 4

Increased metabotropic glutamate receptor subtype 5 availability in human brain after one night without sleep

Katharina Hefti* [1], Sebastian C. Holst* [1,8], Judit Sovago [2], Valérie Bachmann [1,8], Alfred Buck [3,7,8], Simon M. Ametamey [4,7], Milan Scheidegger [5,6], Thomas Berthold [3], Baltazar Gomez-Mancilla [2], Erich Seifritz [5,7,8], and Hans-Peter Landolt [1,7,8]

[1] Institute of Pharmacology & Toxicology, University of Zürich, Switzerland
[2] Novartis Institutes for Biomedical Research, Basel, Switzerland
[3] Division of Nuclear Medicine, University Hospital Zürich, Switzerland
[4] Center for Radiopharmaceutical Sciences of ETH, PSI & USZ, Zürich, Switzerland
[5] Clinic of Affective Disorders and General Psychiatry, Psychiatric University Hospital, Zürich, Switzerland
[6] Institute for Biomedical Engineering, University & ETH Zürich, Switzerland
[7] Neuroscience Center Zürich, University of Zürich & ETH Zürich, Switzerland
[8] Zürich Center for Integrative Human Physiology (ZIHP), University of Zürich, Switzerland

* *These authors contributed equally to this work.*

Published in: ***Biological Psychiatry, 73(2): 161-168, 2013***

Abstract

Background: Sleep deprivation (wake therapy) provides rapid clinical relief in many patients with major depressive disorder (MDD). Changes in glutamatergic neurotransmission may contribute to the antidepressant response, yet the exact underlying mechanisms are unknown. Metabotropic glutamate receptors of subtype 5 (mGluR5) are importantly involved in modulating glutamatergic neurotransmission and neuronal plasticity. The density of these receptors is reduced in the brain of patients with MDD, particularly in brain structures involved in regulating wakefulness and sleep. We hypothesized that prolonged wakefulness would increase mGluR5 availability in human brain.

Methods: mGluR5 binding was quantified with positron emission tomography (PET) in 22 young healthy men who completed 2 experimental blocks separated by one week. Two PET examinations were conducted in randomized, cross-over fashion with the highly selective radioligand, ^{11}C-ABP688, once after 9 hours (sleep control) and once after 33 hours (sleep deprivation) of controlled wakefulness. ^{11}C-ABP688 uptake was quantified in 13 volumes of interest with high mGluR5 expression and presumed involvement in sleep-wake regulation.

Results: Sleep deprivation induced a global increase in mGluR5 binding when compared to sleep control ($p < 0.006$). In anterior cingulate cortex, insula, medial temporal lobe, parahippocampal gyrus, striatum, and amygdala, this increase correlated significantly with the sleep deprivation-induced increase in subjective sleepiness.

Conclusions: This molecular imaging study demonstrates that cerebral mGluR5 availability is increased after a single night without sleep. Given that modulators of mGluR5 have antidepressant properties, further research is warranted to examine whether this mechanism is involved in the potent antidepressant effect of ‘wake therapy’.

Keywords:
Antidepressant; ^{11}C-ABP688; major depression, molecular brain imaging, sleepiness, wake therapy.

Introduction

Sleep and mood regulation are tightly associated. Disrupted sleep is an important diagnostic criterion and risk factor for major depressive disorder (MDD) (Baglioni et al., 2010). Insomnia symptoms often precede the onset of depression by several months, are resistant to treatment, and increase the risk of relapse in remitted patients. Intriguingly, whereas sleep-wake disturbances are highly prevalent in MDD, sleep deprivation (wake therapy) provides rapid clinical relief in many patients (Bunney and Bunney, 2012). Changes in glutamatergic neurotransmission have been proposed to contribute to the antidepressant response (Bunney and Bunney, 2012), but the exact underlying mechanisms are unknown.

Metabotropic glutamate receptors, including metabotropic glutamate receptors of subtype 5 (mGluR5), play an important role in regulating glutamatergic neurotransmission (Sanacora et al., 2008). The density of mGluR5 is reduced in various cortical and subcortical brain regions in patients with MDD when compared with healthy control subjects (Deschwanden et al., 2011). These receptors are present on postsynaptic neurons and glia cells and contribute to long-term depression (LTD), as well as long-term potentiation (Ayala et al., 2009; Izumi and Zorumski, 2012). Moreover, they are involved in sleep-wake related postsynaptic plasticity in rats (Tadavarty et al., 2011) and interact directly or indirectly with different molecular markers of sleep-wake regulation in animals and humans. These markers include Homer 1a (Franken et al., 2001; Maret et al., 2007), fragile X mental retardation protein (FMRP) (Bushey et al., 2009; 2011; Hays et al., 2011), brain-derived neurotrophic factor (BDNF) (Faraguna et al., 2008; Bachmann et al., 2012b), adenosine deaminase (Okada et al., 2003; Bachmann et al., 2012a), and the adenosine A_{2A} receptor (Gallopin et al., 2005; Bodenmann et al., 2012).

Prolonged wakefulness not only affects mood and other day- time functions, including sleepiness, but also elevates sleep need in a recovery night when compared with a baseline night. The most reliable markers of sleep need are the amount of slow wave sleep and electroencephalographic (EEG) slow-wave activity (SWA) in non-rapid-eye-movement (NREM) sleep (Achermann and Borbély, 2011). Functional brain imaging studies consistently suggest that ventromedial prefrontal cortex, basal forebrain, insula, anterior cingulate cortex, striatum, parahippocampal gyrus, precuneus, and other regions are importantly involved in the regulation of slow waves and NREM sleep (Maquet et al., 1997; Dang-Vu et al., 2008; Murphy et al.,

2009). Interestingly, mGluR5 are preferentially expressed in most of these brain regions (Gasparini et al., 2008). In addition, they are important for shaping the EEG slow oscillation in NREM sleep (Blethyn et al., 2006) and synchronized theta mode network activity in wakefulness (Cobb et al., 2000). Based on this convergent evidence for a possible involvement of mGluR5 in sleep-wake regulation, we aimed to quantify mGluR5 availability in rested and sleep-deprived state in humans.

We used the recently developed, selective mGluR5 radioligand, ^{11}C-ABP688, to directly visualize mGluR5 availability in the living human brain (Ametamey et al., 2006; 2007). We quantified in healthy adults ^{11}C-ABP688 uptake in sleep control and sleep deprivation conditions in 13 brain regions with high mGluR5 expression and presumed involvement in sleep-wake regulation. Given the recent observation that mGluR5 density is reduced in depressed patients (Deschwanden et al., 2011) and the suggested role of mGluR5 in sleep-wake related postsynaptic plasticity (Tadavarty et al., 2011), we predicted that prolonged wakefulness may increase mGluR5 availability in the brain.

Methods and Materials

Study participants

The study protocol and all experimental procedures were approved by the cantonal and federal authorities for research on human subjects and carried out in accordance with the Declaration of Helsinki (1964).

Table 1. Demographic characteristics of study participants

Twenty-two male participants completed the study; one participant had to be excluded from positron emission tomography analyses because of strong head movement. German versions and validated German translations of questionnaires were used to assess lifestyle and personality traits. Caffeine consumption was estimated based on the following average caffeine contents per serving: coffee: 100 mg; ceylon or green tea: 30 mg; cola drink: 40 mg (2 dL); energy drink: 80 mg (2 dL); chocolate: 50 mg (100 g). Diurnal preference: Horne-Östberg Morningness-Eveningness Questionnaire (Horne and Ostberg, 1976); daytime sleepiness: Epworth Sleepiness Scale (Bloch et al., 1999); trait anxiety: State-Trait Anxiety Inventory (Spielberger et al., 1970); depression score: Beck Depression Inventory (Beck et al., 1961); personality traits: Eysenck Personality Questionnaire (Francis et al., 2006) and Cloninger's Tridimensional Personality Questionnaire (Weyers et al., 1995).

	Mean ± SD
Age (years)	23.4 ± 2.1
Body-mass index (kg/m^2)	22.1 ± 1.9
Alcohol consumption (drinks/week)	3.5 ± 3.3
Caffeine consumption (mg/day)	153.5 ± 160.9
Diurnal preference	49.7 ± 8.4
Daytime sleepiness	7.1 ± 3.3
Trait anxiety	34.5 ± 7.0
Depression score	3.2 ± 3.8
Eysenck Personality Traits	
Extraversion	8.6 ± 2.8
Neuroticism	3.7 ± 2.8
Lie scale	2.5 ± 1.9
Psychoticism	3.1 ± 1.4
Cloninger Personality Traits	
Novelty seeking	14.7 ± 5.6
Harm avoidance	11.8 ± 4.7
Reward dependence	16.0 ± 5.2

Twenty-two healthy young men completed the 2-week study after giving written informed consent and being extensively screened for medical history and psychological state. Individuals with a significant medical history (e.g., loss of consciousness) or past psychiatric illness were excluded. A prestudy screening night was performed in the sleep laboratory to exclude subjects with unknown sleep disturbances and low sleep efficiency (< 85%). All participants were nonsmokers, right-handed, and abstinent from any medication; denied previous and current illicit drug use; and had not crossed time zones or done shift work during the last 3 months before the experiment. Validated questionnaires to assess lifestyle and demographic characteristics demonstrated the presence of a healthy study sample (Table 1).

Prestudy procedures and experimental protocol

Three days before each study block, participants abstained from caffeine and alcohol and adhered to a 16-hour wake/8-hour sleep schedule. Caffeine, alcohol, and sleep logs; breath-alcohol measurements upon arrival in the laboratory; and wrist-actigraphy were used to verify adherence to these instructions.

All subjects completed two experimental blocks, referred to as sleep control and sleep deprivation conditions. The two conditions occurred in randomized, cross-over fashion, typically 1 week apart, and included polysomnographically recorded adaptation, baseline, and experimental nights (time in bed: 11:30 PM–07:30 AM). In the sleep deprivation condition, the baseline night was followed by 40 hours continuous wakefulness, during which the participants were under constant supervision of the research team. All subjects underwent two positron emission tomography (PET) examinations with ^{11}C-ABP688 to quantify mGluR5 availability in the brain (Division of Nuclear Medicine, University Hospital Zürich). These assessments occurred in random order, either ~9 or ~33 hours after awakening from the baseline night. The time of day of the PET examinations in sleep control (4:36 PM ± 9 minutes) and sleep deprivation (4:34 PM ± 8 minutes) conditions did not differ (p > .4).

Magnetic resonance and PET image acquisition

A T1-weighted, whole-brain, three-dimensional magnetic resonance (MR) image (resolution: 1 x 1 x 1 mm) was obtained for each subject (Philips Achieva 3T whole-body MR unit equipped with transmit/receive head coil; Philips Healthcare, Best, The Netherlands), to exclude morphological abnormalities and as anatomical standard for the quantification of the PET images.

Tracer synthesis and PET brain imaging with ^{11}C-ABP688 using a bolus/infusion protocol were performed as previously described (Ametamey et al., 2006; 2007; Burger et al., 2010; Deschwanden et al., 2011). Catheters were placed into the antecubital veins of each participant's arms, one for tracer injection and one for blood sampling. Venous blood was collected at 42 and 58 minutes after the start of the bolus infusion.

All PET examinations were performed in three-dimensional mode on a DVCT PET/computed tomography scanner (GE Medical Systems, Glattbrugg, Switzerland) or a DSTx PET/computed tomography scanner (B. Braun Medical, Sempach,

Switzerland). A low-dose computed tomography scan was performed before the PET examination for photon attenuation correction. The emission scans were initiated simultaneously with the start of the injection of radioligand using an infusion pump (Perfusor FM, Braun Medical). Injected activity (sleep control: 582.9 ± 23.7 MBq; sleep deprivation: 543.5 ± 19.5 MBq) and mass of cold compound (sleep control: 6.2 ± .7 nmol; sleep deprivation: 6.9 ± .7 nmol) did not differ between the conditions (p_{all} > .39; two-tailed, paired t tests). Of the total activity, 47.6% (sleep control: 294.8 ± 9.9 MBq; sleep deprivation: 288.9 ± 9.8 MBq) was injected as a bolus over 2 minutes (K_{bol} = 53 min) and the rest was continuously infused over 58 minutes (sleep control: 283.7 ± 9.6 MBq; sleep deprivation: 278.0 ± 9.5 MBq). Specific activity of ^{11}C-ABP688 at the end of syntheses was also very similar in sleep control (132.6 ± 15.4 GBq/μmol; range: 71–289 GBq/μmol) and sleep deprivation conditions (109.0 ± 13.1 GBq/μmol; range: 61–243 GBq/μmol). Twenty frames were collected during the 60-minute protocol (10 x 60 sec and 10 x 300 sec). The images were reconstructed using filtered back projection and displayed over 47 transaxial slices. Using a 128 x 128 matrix, the resulting voxel size was 2.3 x 2.3 x 3.2 mm.

Subjects were instructed to not fall asleep during image acquisition and the EEG was simultaneously recorded, according to established procedures (Bodenmann et al., 2009a; Bachmann et al., 2012a), to verify wakefulness during the PET examinations. As soon as sleep-like EEG activity was noted, subjects were alerted via an intercom. Direct contact with them was avoided, to minimize movement artifacts.

Image processing and quantification

Image processing consisted of within-subject motion correction by realigning the average of frames 17 to 19 to the average of frames 2 to 10 (rigid matching) and spatial normalization of averaged frames 17 to 19 to the Montreal Neurological Institute template brain for definition of volumes of interest (VOI). The averaged frames 17 to 19 of the PET images were then co-registered to the corresponding MR image to delineate the cerebellum (Burger et al., 2010). These steps were performed with the PMOD software package, version 3.1 (PMOD Technologies, Zürich, Switzerland). Standard VOIs for the Montreal Neurological Institute template brain available in PMOD were used to measure radioactivity concentration in the normalized PET images (Tzourio-Mazoyer et al., 2002). Because PMOD provides no single VOI for cerebellum, this region was defined manually in each subject on the

MR image and subsequently transferred to the corresponding PET images to measure radioactivity concentration in this region. Tissue time activity curves (TAC) were generated for cerebellum, anterior cingulate cortex, superior frontal cortex, putamen, and thalamus in both hemispheres to confirm that steady state of receptor binding was reached in frames 17 to 19 (45–55 min) of image acquisition. The TAC for cerebellum and anterior cingulate cortex in two representative individuals in sleep control and sleep deprivation conditions are illustrated in the supplements (see Appendix 2).

The average radioactivity concentration between 45 and 55 minutes was calculated in each VOI ($C_{t[VOI]}$). Because some venous blood metabolite analyses were unreliable due to technical difficulties, regional V_t values could not be obtained in all subjects. Instead, quantification of the PET images was done by dividing the regional radioactivity concentration values with the corresponding value in the cerebellum ($C_{t[Cb]}$) to obtain V_{norm} ($V_{norm} = C_{t[VOI]} / C_{t[Cb]}$). This method was successfully used in previous studies to quantify mGluR5 availability in the brain (Burger et al., 2010; Deschwanden et al., 2011). Furthermore, a preclinical study showed that ^{11}C-ABP688 binding in the cerebellum is negligible and that this region can thus be used as a reference region (Elmenhorst et al., 2010). To address the question whether sleep deprivation changed ^{11}C-ABP688 binding in the cerebellum, we calculated the nondisplaceable volume of distribution (V_{ND}) in sleep control and sleep deprivation conditions in those nine study participants in whom reliable plasma radioactivity concentration and metabolite concentrations were available. These analyses confirmed that ^{11}C-ABP688 binding in the cerebellum was not changed after sleep deprivation when compared with the control condition (control: V_{ND} = 1.98 ± .14; sleep deprivation: V_{ND} = 2.18 ± .16; p = .19, two-tailed paired t test).

Behavioral and cognitive effects of sleep deprivation

To document that sleep deprivation was successful, validated questionnaires were employed before each PET scan to assess subjective sleepiness (Karolinska Sleepiness Scale) (Åkerstedt and Gillberg, 1990), state anxiety (State Trait Anxiety Inventory) (Spielberger et al., 1970), and affective state (Profile of Mood States) (McNair et al., 1971). Approximately 3 hours before each scan, the subjects also completed a cognitive test session consisting of the psychomotor vigilance task (PVT) (Dinges and Powell, 1985) and the Deese-Roediger-McDermott false memory paradigm (Deese, 1959; Roediger and McDermott, 1995). To approach a normal

distribution, mean reaction time (RT) on the PVT was expressed as speed (1/RT) and the number of lapses (RT > 500 ms) was transformed by ($\sqrt{x} + \sqrt{[x + 1]}$).

Cortisol concentration in saliva

Immediately before each PET examination, all study participants provided a saliva sample to determine the cortisol concentration as a measure of acute stress. Unstimulated saliva was collected by placing a salivette (Sarstedt, Sevelen, Switzerland) under the tongue and keeping the head slightly inclined for 1 to 2 minutes. The samples were stored at -20 ^{o}C until the biochemical analysis took place. The saliva was centrifuged at 3000 rpm for 5 minutes before free cortisol was analyzed using an immunoassay with time-resolved fluorescence detection (Dressendörfer et al., 1992).

Statistical analyses

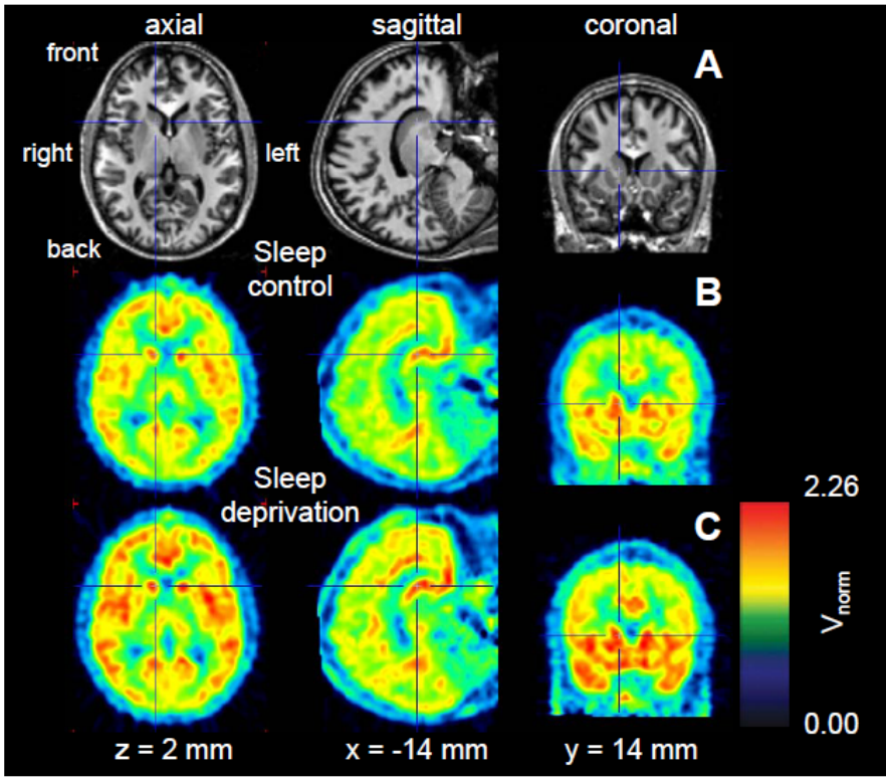
All statistical analyses were performed with SAS 9.1 software (SAS Institute, Cary, North Carolina). Mixed-effect analysis of variance models included the factors condition (sleep control, sleep deprivation), region (13 VOIs), and hemisphere (left, right), as well as their interactions. Two-tailed, paired t tests were conducted to localize significant differences. To limit the number of comparisons and to control for type I errors, analyses of PET data were restricted to the predefined VOIs and the significance level was set at $\alpha < .0038$ (Bonferroni correction: $\alpha = .05/13$). To estimate the possible associations between the effects of sleep deprivation on mGluR5 availability and changes in behavioral and cognitive variables, regression analyses were performed and Spearman rank correlation coefficients were calculated. If not stated otherwise, only significant results are reported.

Results

Regional uptake of ^{11}C-ABP688 in human brain

Axial, sagittal, and coronal views of ^{11}C-ABP688 binding in the brain of one single individual in sleep control and sleep deprivation conditions are presented in Figure 1. Consistent with previous studies (Ametamey et al., 2007; DeLorenzo et al., 2011), regional uptake of ^{11}C-ABP688 reflected the known distribution of mGluR5 with most pronounced binding in anterior cingulate, insula, medial temporal lobe, medial prefrontal cortex, striatum, and amygdala. Lower radioligand binding was present in thalamus and substantially lower radioligand binding was present in cerebellum, which was used to calculate the normalized volumes of distribution (V_{norm}). Visual inspection of TAC in distinct brain regions confirmed that a steady state of ^{11}C-ABP688 uptake was reached 45 minutes after tracer injection in all participants and scans.

Figure 1. Axial, sagittal and coronal views of ^{11}C-ABP688 binding in a representative individual.



(**A**) Magnetic resonance image (MRI) template for anatomical reference. (**B**) Color-coded normalized volumes of distribution (V_{norm}) of ^{11}C-ABP688 after ~ 9 hours of wakefulness (sleep control condition). (**C**) Color-coded V_{norm} of ^{11}C-ABP688 after ~ 33 hours of wakefulness (sleep deprivation condition). The crosshair was placed in the right caudate nucleus (coordinates according to the Montreal Neurological Institute brain atlas: -14, 14, 2).

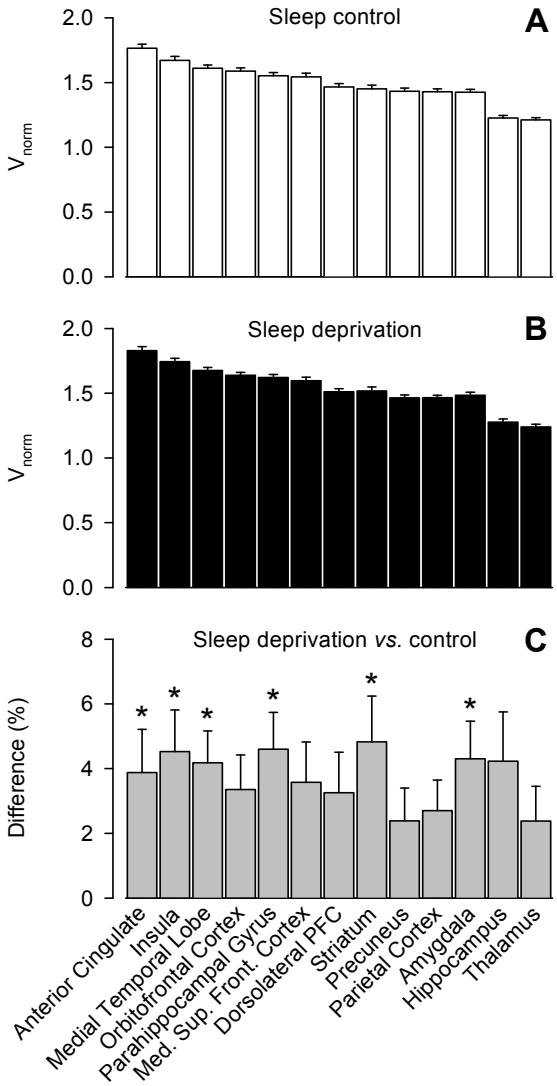


Figure 2. Regional differences in mGluR5 density and effect of sleep deprivation. (**A**) Normalized volumes of distribution (V_{norm}) of ^{11}C-ABP688 uptake in 13 regions of interest after ~ 9 hours of wakefulness (sleep control). (**B**) V_{norm} after ~ 33 hours of wakefulness (sleep deprivation). (**C**) Percent difference in V_{norm} between sleep deprivation and sleep control conditions. The two measurements occurred in random order and were separated by one week. Data represent means + 1 SEM (n = 21). Asterisks indicate significant differences between sleep deprivation and sleep control conditions ('condition': $p < .0038$). Med. Sup. Front. Cortex = medial superior frontal cortex; PFC = prefrontal cortex.

Sleep deprivation increases functional mGluR5 availability in human brain

Regional V_{norm} values of ^{11}C-ABP688 in sleep control and sleep deprivation conditions are shown in Figure 2. Prolonged wakefulness increased global V_{norm} by 3.5 ± 1.1% when compared with control (1.50 ± .02 vs. 1.55 ± .02, $p < .006$). Mixed-model analysis of variance with the factors condition, region (VOIs), and hemisphere revealed highly significant main effects of condition [$F(1,20) = 184.5$, $p < .0001$] and region [$F(25,500) = 257.6$, $p < .0001$], yet no significant effect of hemisphere [$F(1,20) = .1$, $p > .7$]. Thus, the data of left and right hemispheres were averaged. The increase in mGluR5 binding was significant in anterior cingulate cortex (3.9 ± 1.3%, $p < .0006$), insula (4.5 ± 1.3%, $p < .0001$), medial temporal lobe (4.2 ± 1.0%, $p < .0005$), parahippocampal gyrus (4.6 ± 1.1%, $p < .0002$), striatum (4.8 ± 1.4%, $p <$

.0003), and amygdala (4.3 ± 1.2%, $p < .002$). No VOI showed a reduction in ^{11}C-ABP688 binding after sleep deprivation when compared with control.

Increase in ^{11}C-ABP688 binding correlates with increase in subjective sleepiness

Sleep deprivation impaired subjective state and cognitive performance. Sleepiness, state anxiety, fatigue, lapses on the PVT, and false alarms on the Deese-Roediger-McDermott false memory paradigm were increased after prolonged wakefulness when compared with control (Table 2). By contrast, vigor was reduced and PVT reaction times were prolonged.

Table 2. Behavioral and cognitive effects of sleep deprivation.

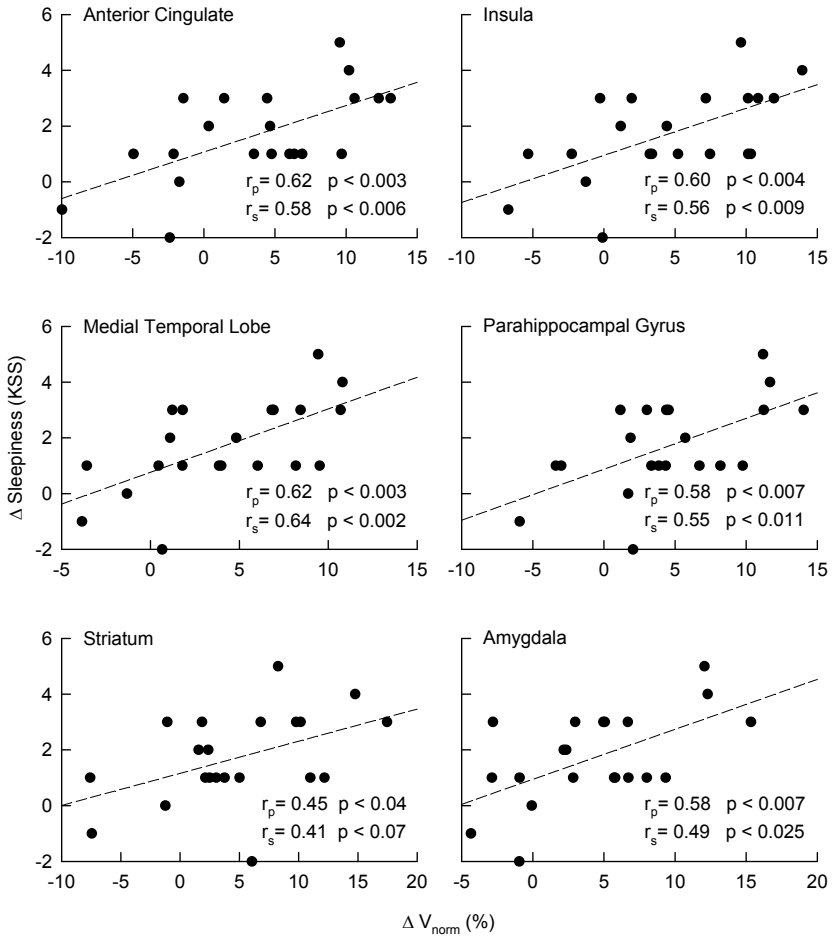
	Sleep control	Sleep deprivation	*p* <
Karolinska Sleepiness Scale	3.13 ± 0.28	5.04 ± 0.43	**0.001**
State Anxiety Inventory	36.45 ± 2.07	40.73 ± 2.47	**0.001**
Profile of Mood States (POMS)			
Fatigue	5.77 ± 1.49	13.86 ± 2.22	**0.001**
Vigor	18.54 ± 1.35	10.54 ± 1.38	**0.001**
Depression/Anxiety	3.32 ± 1.42	4.14 ± 2.08	0.506
Irritability	0.91 ± 0.53	1.68 ± 0.72	0.284
Psychomotor vigilance task			
Mean reaction speed (s^{-1})	3.79 ± 0.07	3.50 ± 0.07	**0.001**
Lapses (transformed)	2.29 ± 0.25	4.71 ± 0.62	**0.001**
Deese-Roediger-McDermott Paradigm			
False alarms	5.25 ± 0.99	8.40 ± 0.86	**0.001**
False memory	12.25 ± 0.55	13.80 ± 0.68	0.067
Hit rate	40.35 ± 1.51	41.15 ± 1.80	0.638

Values represent means ± SEM (n = 22). *p* values refer to 2-tailed, paired *t* tests. Significant differences between sleep control and sleep deprivation conditions are highlighted in bold.

Correlation analyses between the sleep deprivation-induced change in subjective sleepiness and the percent change in mGluR5 availability revealed consistent associations in all brain regions showing a significant increase in ^{11}C-ABP688 binding. In other words, those subjects who were most affected by sleep deprivation exhibited the largest increase in mGluR5 binding in anterior cingulate cortex, insula, medial temporal lobe, parahippocampal gyrus, striatum, and amygdala (Figure 3). A similar association was also found for fatigue on the Profile of Mood States questionnaire and mGluR5 availability in medial temporal lobe ($r_p = .43$, $p = .053$; $r_s = .45$, $p < .04$; n = 21). The other behavioral and cognitive effects of sleep deprivation

revealed no consistent correlation with the changes in mGluR5 binding in the significant VOIs.

Figure 3. Relationship between the individual subjects' difference in subjective sleepiness and the difference in mGluR5 availability between sleep deprivation and sleep control conditions.



Individual percent changes in normalized volumes of distribution (V_{norm}) of ^{11}C-ABP688 in those brain regions that showed a significant increase after sleep deprivation are illustrated. A linear regression line was fitted through the 21 individual data points. KSS = Karolinska Sleepiness Scale; r_p = Pearson's product-moment correlation coefficient; r_s = Spearman rank correlation coefficient.

Cortisol concentration in saliva

Prolonged wakefulness tended to slightly increase salivary cortisol concentration when compared with the sleep control condition (9.4 ± .6 vs. 7.0 ± .6 mmol/L, $p < .08$). The individual changes in salivary cortisol were not associated with individual changes in mGluR5 binding.

Discussion

This in vivo molecular imaging study in humans reveals the first evidence for increased functional cerebral mGluR5 availability after prolonged wakefulness. After a single night without sleep, binding of ^{11}C-ABP688 was significantly increased on a global level and in distinct brain regions that were previously shown to reflect physiological changes after sleep deprivation (for recent review, see (Dang-Vu et al., 2010)). These effects of short-term sleep loss are intriguing. The expression of G-protein coupled receptors on the cell surface is tightly regulated and provides a powerful mechanism of signal amplification (Nelson and Cox, 2008). A recent study demonstrated that regional ^{11}C-ABP688 binding may reflect mGluR5 protein expression (Deschwanden et al., 2011). Because mGluR5 density is reduced in major depression (Deschwanden et al., 2011), our findings suggest that changes in functional mGluR5 availability may be involved in the rapid antidepressant effect of sleep deprivation in MDD patients.

The mGluR5 are mostly expressed on postsynaptic membranes of neurons and astrocytes in corticolimbic areas of the brain, including medial-prefrontal and orbitofrontal cortex, cingulate, striatum, amygdala, and hippocampus (Gasparini et al., 2008). They may be very well positioned to integrate and modulate the expression of established molecular markers of wakefulness and sleep. Indeed, mGluR5 interact either directly or indirectly with Homer1a, FMRP, BDNF, adenosine deaminase, and A_{2A} receptors. Convergent evidence obtained from studies in invertebrates, rodent models, and humans strongly indicate that all these molecules play a causal role in sleep-wake regulation (Urade et al., 2003; Maret et al., 2007; Faraguna et al., 2008; Bushey et al., 2009; 2011; Bodenmann et al., 2012; Bachmann et al., 2012a; 2012b).

It was previously suggested that sleep is crucial for protecting and recovering the brain from increased intracellular calcium concentrations imposed by prolonged wakefulness (Maret et al., 2007). The immediate early gene Homer1a is a proposed core molecular marker of sleep homeostasis, i.e., the sleep-wake dependent facet of sleep regulation (Maret et al., 2007). Homer1a selectively uncouples mGluR5 from effector targets in the membrane of the postsynaptic density and attenuates the mGluR5-mediated rise in intracellular calcium levels (Kammermeier and Worley, 2007). The interaction between Homer1a and mGluR5 is necessary for mGluR5-dependent synaptic LTD (Ronesi and Huber, 2008) and may promote synaptic

changes during sleep (Maret et al., 2007). Interestingly, Homer1a was shown to alter mGluR5 signaling without inducing large-scale changes in mGluR5 distribution (Kammermeier and Worley, 2007).

Not only Homer1a, but also FMRP, interacts with mGluR5. A genetic defect in the X chromosome-linked human *FMR1* (fragile X mental retardation 1) gene encoding FMRP gives rise to increased mGluR5 signaling and Fragile X syndrome (FXS), the most common form of inherited mental retardation and a leading cause of autism. Brain slices of *FMR1* knockout mice, an established model of FXS, show enhanced mGluR5-mediated synaptic LTD (Huber et al., 2002). In vitro as well as in vivo, these mice show prolonged spontaneous UP-states, which predominantly occur in slow wave sleep. This altered neocortical rhythmic activity is due to enhanced mGluR5 signaling (Hays et al., 2011). Sleep-wake regulation was studied in Drosophila mutants carrying dFmr1 loss-of-function (amorphs) and gain-of-function (hypermorphs) mutations (Bushey et al., 2009). The dFmr1 amorphs were long sleepers, whereas dFmr1 hypermorphs were short sleepers. A recent study further demonstrated that dFmr1 is important for sleep-dependent synaptic normalization (Bushey et al., 2011).

Also, interactions of mGluR5 with BDNF and adenosinergic neurotransmission may be important for sleep-wake regulation. Expression of BDNF in cerebral cortex is high during wakefulness, low during sleep, and increased after sleep deprivation (Cirelli and Tononi, 2000). Cortical injection of BDNF to awake animals promotes synaptic strength and enhances SWA in subsequent NREM sleep. This effect is reversible and opposite to the changes in local SWA after pharmacologic inhibition of BDNF-tyrosine-kinase-b-receptor stimulation (Faraguna et al., 2008). Pharmacologic activation of mGluR5 induces BDNF expression in rat cortical neurons and glia cells (Legutko et al., 2006; Viwatpinyo and Chongthammakun, 2009). It may, thus, be speculated that enhanced mGluR5-induced BDNF secretion after prolonged wakefulness contributes to the antidepressant response to sleep deprivation. Indeed, patients suffering from MDD typically exhibit reduced serum BDNF levels, which may normalize after antidepressant therapies (Nagahara and Tuszynski, 2011).

The facilitatory action of BDNF on hippocampal long-term potentiation requires adenosine A_{2A} receptor activation by endogenous adenosine (Fontinha et al., 2008). A primary role for adenosine and adenosine receptors in sleep regulation is well established in animals and humans (Basheer et al., 2004; Landolt, 2008). In an

approach similar to the present study, it was recently shown that sleep deprivation increases A_1 receptor binding in human brain (Elmenhorst et al., 2007). Nevertheless, accumulating evidence indicates that also A_{2A} receptors contribute to sleep-wake regulation (Gallopin et al., 2005; Bodenmann et al., 2012). These receptors are co-localized with mGluR5, dopamine D_2, and N-methyl-D-aspartate receptors and functionally interact in vitro and in vivo (Ferré et al., 2002; Tebano et al., 2005; D'Ascenzo et al., 2007; Rebola et al., 2008). Importantly, a recent PET study showed that one night without sleep reduced dopamine $D_{2/3}$ receptor availability in the ventral striatum by roughly 5% (nondisplaceable binding potential of ^{11}C-raclopride: 2.80 ± .37 vs. 2.95 ± .37) (Volkow et al., 2012). After the same experimental procedure, we found an increase in mGluR5 availability in the striatum by 4.8 ± 1.4% ($p < .0003$). Given these results, it is intriguing to note that mGluR5 and D_2 receptor signaling in the striatum functionally counteract each other by means of intramembrane interactions (Ferré et al., 2007). The almost identical magnitude of sleep-deprivation induced changes in mGluR5 and $D_{2/3}$ receptor availability and the significant correlation in both studies with increased sleepiness after the night without sleep strongly support the notion that the reported changes are real and have more than mere statistical significance.

Because plasma samples were not available for calculating regional V_t in all subjects, the cerebellum was used to calculate V_{norm}. The density of mGluR5 in this region is unlikely to produce a specific binding signal with PET. Preclinical and clinical studies demonstrated that the cerebellum is suitable to quantify ^{11}C-ABP688 binding in the brain (Burger et al., 2010; Elmenhorst et al., 2010; Deschwanden et al., 2011). Another possible limitation of our study may be due to the fact that two different PET scanners were employed for examining the subjects. Because each volunteer was examined on the same scanner in sleep control and sleep deprivation conditions and a within-subject design was used, it is improbable that differences between PET scanners have biased the findings.

In conclusion, the current observations indicate that mGluR5 may be involved in the effects of sleep deprivation. To corroborate this notion and to address the question whether changes in glutamate concentration could underlie the current observations, multimodal imaging studies combining PET and magnetic resonance spectroscopy are warranted to simultaneously measure functional mGluR5 availability and glutamine/ glutamate levels in the brain. The mGluR5 has been implicated in various central nervous system pathologies, and pharmacologic agents

targeting this receptor currently provide promising future treatments for psychiatric and neurological disorders, including schizophrenia, anxiety, FXS, substance abuse, and drug withdrawal (Gasparini et al., 2008; undefined author and Conn, 2010). Studies also suggest that mGluR5 agonists have antidepressant-like properties (Sanacora et al., 2008). Blockade of glutamatergic neurotransmission with the N-methyl-D-aspartate receptor antagonist ketamine and sleep deprivation therapy both rapidly reverse depression in a large subgroup of patients (Bunney and Bunney, 2012). Given the reduced mGluR5 density in depressed patients (Deschwanden et al., 2011) and the enhanced functional mGluR5 availability after prolonged wakefulness, it is tempting to hypothesize that mGluR5 are involved in the rapid mood-enhancing effects of sleep deprivation. Because the neurobiology of depressed patients is likely to differ from healthy volunteers, future research is needed to confirm this possible mechanism in patients with depression. Such an approach may lead to novel treatments of depression and other mental disorders, which, according to the World Health Organization, will become the biggest health burden on society within the next 20 years.

Aknowledgments

This work was supported by Swiss National Science Foundation Grant #320030-135414, National Center of Competence in Research Neural Plasticity and Repair, and Novartis Foundation for Biomedical Research. ABP688 and its precursor were provided by Novartis Pharma AG.

We cordially thank all members of the Section of Chronobiology and Sleep Research, Institute of Pharmacology and Toxicology, University of Zürich, and the Division of Nuclear Medicine, University Hospital Zürich, who helped with data collection and analyses.

Drs. Sovago and Gomez-Mancilla are employees of Novartis Pharma AG (Basel), which is developing and testing drugs targeting the metabotropic glutamate receptor subtype 5. Ms. Hefti, Mr. Holst, Dr. Bachmann, Dr. Buck, Dr. Ametamey, Mr. Scheidegger, Mr. Berthold, Dr. Seifritz, and Dr. Landolt report no biomedical financial interests or potential conflicts of interests.

Chapter 5

Human electroencephalographic markers of sleep need is associated with cerebral mGluR5 availability and modulated by the fragile X gene

Sebastian C. Holst[1,8], Katharina Hefti[1], Judit Sovago[2], Alfred Buck[3,9], Simon M. Ametamey[4,9], Milan Scheidegger[5], Roland Dürr[1], Alessandra Baumer[6], Baltazar Gomez-Mancilla[2], Erich Seifritz[7,8,9], and Hans-Peter Landolt[1,8,9]

[1] Institute of Pharmacology & Toxicology, University of Zürich, Switzerland
[2] Novartis Institutes for Biomedical Research, Basel, Switzerland
[3] Division of Nuclear Medicine, University Hospital Zürich, Switzerland
[4] Center for Radiopharmaceutical Sciences of ETH, PSI & USZ, Zürich, Switzerland
[5] Institute for Biomedical Engineering, University of Zürich & ETH , Zürich, Switzerland
[6] Institute of Medical Genetics, University of Zürich, Switzerland
[7] Clinic of Affective Disorders and General Psychiatry, Psychiatric University Hospital, Zürich, Switzerland
[8] Zürich Center for Integrative Human Physiology (ZIHP), University of Zürich, Switzerland
[9] Neuroscience Center Zürich, University of Zürich & ETH Zürich, Switzerland

Manuscript in preparation

Abstract

Although sleep is hypothesized to play an important role for maintaining neuronal homeostasis, in vivo molecular mechanisms of sleep remain only poorly understood. In Drosophila models of Fragile X mental retardation (FXS), the expression of fragile X mental retardation protein (FMRP) is reduced, whereas sleep need is strongly enhanced. On the other hand selective inhibitors of metabotropic glutamate receptors of subtype 5 (mGluR5) rescue the FXS phenotype in mice. Therefore FMRP and mGluR5 can be considered opposing regulators of neuronal plasticity and used to investigate molecular mechanism of sleep in humans. Here we combine genetic analysis of the *FMR1* gene with nocturnal EEG recordings and positron emission tomography scans of mGluR5 availability during a randomized cross over sleep deprivation study, to further elaborate on the relationship between FMRP, mGluR5 and EEG markers of sleep need. 26 healthy men underwent the two experimental blocks were mGluR5 was quantified using the highly selective radioligand ^{11}C-ABP688, following 9 and 33 hours of wakefulness.

Our data reveal a strong association between global mGluR5 availability and EEG markers of sleep need. Especially EEG slow oscillations (<1 Hz) were found to be highly correlated with mGluR5 availability in both baseline and sleep deprived conditions. Genetic analysis revealed that healthy *FMR1* odd CGG carriers have increased FMRP expression, associated with a damped response to sleep deprivation observed by a stagnant global mGluR5 availability, EEG slow oscillations and slow wave activity. Our data propose the mGluR5 to be a molecular marker for sleep need in humans and to play an important role for especially the generation of EEG slow oscillations. Furthermore, our data suggest that the *FMR1* genotype modulate mGluR5 availability and affect EEG makers of sleep need in healthy adult men.

Keywords:
FMRP; ^{11}C-ABP688; molecular brain imaging; sleep homeostasis; slow oscillations.

Introduction

Synaptic plasticity underlying memory and learning, is modulated by the availability and activation of ionotropic glutamate receptors such as a-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) and n-methyl-d-aspartate receptors (NMDARs) (Malenka and Bear, 2004; Kauer and Malenka, 2007). Metabotropic glutamate receptors of subtype 5 (mGluR5) activate phosporlipase C which catalyzes the second messenger's inositol triphosphate (IP3) and diacyl glycerol (DAG) enhancing downstream signal amplification and protein synthesis (Nelson and Cox, 2008). mGluR5 thereby indirectly control NMDAR and AMPAR expression (Oliet et al., 1997; Collingridge et al., 2004). The mGluR5 are mainly post- and extra-synaptic receptors involved in long-term depression (LTD) and to some extent long-term potentiation (LTP) (Ayala et al., 2009; Izumi and Zorumski, 2012). Furthermore, the mGluR5 have been linked to the generation of slow-oscillation during NREM sleep (Blethyn et al., 2006), sleep specific brain-oscillations which have been suggested to synchronize cortical synaptic processes (Buzsáki and Draguhn, 2004) and as such have been associated with memory enhancements (Marshall et al., 2006).

Stimulation of mGluR5 results in the synthesis of Fragile X mental retardation protein (FMRP), a RNA binding protein, suggested to regulate neuronal mRNA trafficking and translation at synapses (Bagni and Greenough, 2005; Ben A Oostra and Willemsen, 2009). Evidence suggests that FMRP and mGluR5 are functionally opponent; activation of mGluR5 initiates protein synthesis whereas FMRP suppress it (Dölen et al., 2007; Dölen and Bear, 2008). Indeed, the loss of FMRP strongly inhibits normal brain functions and leads to abnormal spine formation, as observed in Fragile X syndrome (FXS) (Irwin et al., 2001). FXS and derivative changes in FMRP have been extensively studied in animal models. Brain FMRP levels in drosophila has been shown to increase with time awake, and to be inversely linked to sleep duration (Bushey et al., 2009). Overexpression of FMRP in the fly model revealed reduced neuronal plasticity in response to sleep deprivation and an overall reduced sleep duration (Bushey et al., 2011). *FMR1* knock-out mice also show signs of increased synaptic activity and spine formation (Nimchinsky et al., 2001), which can also be observed by an increased associations between mGluR5 and the immediate early gene Homer1a (Ronesi et al., 2012), a pronounced marker of sleep need (Franken et al., 2001; Maret et al., 2007). Treatment of *FMR1* knock.-out mice with selective mGluR5 inhibitors rescues the FXS phenotype and reduces hyperactivity

(Michalon et al., 2012). Selective mGluR5 positive allosteric modulators on the other hand, increase locomotors activity and reduce delta sleep, a marker of sleep need in mice (Parmentier-Batteur et al., 2013). Moreover mGluR5 have been shown to interact with brain-derived neurotropic factor (BDNF) (Faraguna et al., 2008; Bachmann et al., 2012b), adenosine deaminase (Okada et al., 2003; Bachmann et al., 2012a) and adenosine A_{2A} receptors (Gallopin et al., 2005; Bodenmann et al., 2012) all of which are believed to be importantly involved in sleep wake regulation.

Taken together, evidence suggests a strong interaction between mGluR5, FMRP and sleep need, at least in the animal models. In humans, FXS is the most common form of inherited mental retardation, which is caused by a 5' $(CGG)_n$ repeat expansion in the Fragile X mental retardation 1 (*FMR1*) gene. Repeat expansions above 200 CCG repeats result in DNA hyper methylation and complete gene silencing (Cummings and Zoghbi, 2000). As defined by The American College of Medical Genetics (Maddalena et al., 2001), premutation $(CGG)_n$ expansions of 54-199 repeat, have been linked to milder inherited disorders such as fragile X tremor ataxia syndrome (FXTAS) and premature ovarian failure. Indeed, the premutation range is associated with increased mRNA levels (Allen et al., 2004), yet decreased FMRP expression (Tassone et al., 2000; Peprah et al., 2009). Furthermore specific AGG interruptions in the $(CGG)_n$ sequence increase repeat stability and reduce the risk of maternal premutation carriers transmitting a full mutation to their offspring (Yrigollen et al., 2012). Nevertheless, the AGG interruptions do not appear to affect FMRP expression levels in premutation carriers (Yrigollen et al., 2011).

Among healthy individuals (5-45 CGG repeats), little is known about how *FMR1* expression is regulated or whether the number of CGG repeats play any significant role. Indeed, studies examining both premutation and healthy carriers have shown a linear increase in *FMR1* transcription levels with increased CGG repeat number, however, within the healthy CGG repeat range alone, no association was found (Allen et al., 2004). One important factor for protein expression namely stemloop formation or more generally RNA folding patterns (Malys and McCarthy, 2011), have not systematically been investigated. Previously, *FMR1* gene expression was linked to the formation and stability of RNA hairpins, which are known to form complex 3-dimensional structures (Darlow and Leach, 1995; 1998a; Mirkin, 2007; Zumwalt et al., 2007). Nevertheless, Darlow and Leach could show in vitro, that odd number

CGG repeats are associated with the formation of more stable hairpins, when compared to even (Darlow and Leach, 1995; 1998b; 1998a).

Among healthy male subjects, we could recently show that prolonged wakefulness increase brain mGluR5 availability and how increased mGluR5 availability positively correlates with increased sleepiness (Hefti et al., 2013) (Chapter 4). To investigate the association between mGluR5 and sleep-wake regulation further, we here examine how the increase in mGluR5 availability following sleep deprivation correlates with nocturnal and waking EEG markers of sleep need. Furthermore, we investigate potential roles for the *FMR1* $(CGG)_n$ expansion in healthy men, and thereby how sleep wake regulation is modulated by FMRP expression and interacts with global mGluR5 availability.

Methods

Study participants and pre-experimental procedure

In total Twenty-six healthy young men, three more than previously published, completed this 2-week study after giving their written informed consent and a blood sample for genetic analysis. As describe previously, all subjects fulfilled strict inclusion and exclusion criteria related to sleep quality, psychological wellbeing, medication and drug intake (Hefti et al., 2013). Furthermore, three days before experimental blocks, participants did not consume caffeine or alcohol and had to stringently adhere to 8 h sleep and 16 h wake periods, monitored by wrist-actigraphy and breath-alcohol measurements.

Genotyping

Genotypes were determined using the MJ Research PTC-225 thermal PCR cycler (MJ Research/Bio-Rad, Reno, NV) at an annealing temperature of 67 °C with the *FMR1* E1-forward primer 5'-ctccgtttcggtttcacttc-3' and *FMR1* E1-reverse primer 5'-atcttctcttcagccctgct-3', by HOT FIREPol DNA Polymerase. Sequencing was performed using the Sanger chain-termination method (Sanger et al., 1977) with an ABI PRISM 3100 (16 capillaries) genetic analyzer (Applied Biosystems Inc., Foster City, CA). Genomic DNA was extracted from 3 ml fresh EDTA-blood (Wizard Genomic DNA Purification Kit, Promega, Madison, WI). Based on the number of CGG repeats in the *FMR1* gene, participants were grouped as either "odd" (n = 12) or "even" (n = 14) carriers.

Experimental protocol and image processing

All experimental procedures were conducted in accordance with the declaration of Helsinki (1964) and approved by the cantonal and federal authorities for research on human subjects in Zurich, Switzerland.

The experimental protocol was explained in detail previously (Hefti et al., 2013). In short, subjects underwent two experimental conditions of sleep control (sc) and sleep deprivation (sd) in a randomized cross-over fashion. Eight hour sleep episodes were recorded with polysmonography. After ~9 (16:39 PM ± 8.23 min) and ~33 (16:30 PM ± 7.40 min) hours of wakefulness, a 60 minute positron emission tomography (PET) scan were carried out at the Division of Nuclear Medicine, University Hospital Zürich, with the highly selective mGluR5 radioligand ^{11}C-ABP688. Due to technical problems, three subjects had to be excluded from PET analyses. Tracer synthesis and three-

dimensional PET brain imaging was carried out using the previously validated bolus/infusion protocol (Ametamey et al., 2006; 2007; Burger et al., 2010; Deschwanden et al., 2011), on two intra-individually assigned GE Healthcare PET scanners (DVCT PET/CT or DSTx PET/CT scanner) with 2.3 x 2.3 x 3.2 mm voxel resolution (Hefti et al., 2013). Image processing consisted of within-subject rigid matching motion correction as well as spatial normalization of averaged frames 17 to 19 (45-55 min) to the Montreal Neurological Institute (MNI) template brain in PMOD software package, version 3.1 (PMOD Technologies, Zürich, Switzerland). Furthermore whole-brain T1-weighted 3D-MR images (Philips Achieva 3T whole-body MR unit equipped with transmit/receive head coil; Philips Healthcare, Best, The Netherlands) were obtained to exclude subjects with morphological abnormalities and in order to co-register MR and PET images, allowing for accurate cerebellum delineation. Quantification of the PET images was done by dividing regional radioactivity concentration values with the corresponding value in the cerebellum ($C_{t[Cb]}$) to obtain V_{norm} ($V_{norm} = C_{t[VOI]} / C_{t[Cb]}$). Here we investigate not only changes in global mGluR5 availability but also regional specific changes for 13 regions with high mGluR5 expression and presumed involvement in sleep-wake regulation (Hefti et al., 2013).

Comparison of injected radioactivity dose (sc: 582 ± 18.2, sd: 568 ± 18.5 MBq/ml), end of synthesis specific activity (sc: 127 ± 14.5, sd: 107 ± 11.4 GBq/μmol), mass of cold compound (sc: 6.39 ± 0.65, sd: 6.92 ± 0.59 nmol) and cerebellar standard uptake values (sc: 826 ± 29.7, sd: 788 ± 24.9 g/ml) revealed no difference between the conditions (p_{all}>0.1, paired t tests) nor between *FMR1* odd even genotypes as measured by two-way repeated measure ANOVA's ("condition": $F_{all} < 2.73$, $p_{all} > 0.11$; "genotype": $F_{all} < 1.13$, $p_{all} > 0.29$; "genotype x condition": $F_{all} < 1.93$, $p_{all} > 0.17$).

EEG and polysomnographic recordings

Continuous polysomnographic EEG were recorded during PET scans and all experimental nights. EEG, electrooculogram (EOG), submental electromyogram (EMG), and electrocardiogram (ECG) were recorded with Rembrandt Datalab (Version 8; Embla Systems, Planegg, Germany) using an Artisan polygraphic amplifier (Micromed, Mogliano Veneto, Italy). During PET image acquisition, subjects were instructed not to fall asleep and in case of sleep-like EEG activity, were alerted via an intercom.

The Analog EEG signals were sampled at 256 Hz and conditioned by high-pass (-3 dB at 0.15-0.16 Hz) and low pass filtering (-3 dB at 67.2 Hz). The EEG was recorded from 1 referential (C3A2) and 8 bipolar derivations (only data from the C3A2 derivation is reported here). Sleep stages were visually scored according to standard criteria (Rechtschaffen and Kales 1968) in 20 s epochs. Four-second EEG spectra (fast Fourier transform [FFT] routine, Hanning window, 0.25 Hz resolution; 0-20 Hz) were calculated with MATLAB (MathWorks Inc., Natick, MA) and averaged over 5 consecutive epochs matched with scored sleep stages. Arousal and movement artifacts were visually identified and eliminated. Nocturnal and PET scan power spectra recordings were analyzed in 0.25 Hz resolution for both NREM (stages 1 to 4) and REM sleep as the average of all artifact-free 20 s values. Classical EEG spectral power bands for SWA (0.5-4.5 Hz), Theta (4-8 Hz), Alpha (8-12 Hz), Spindle (12-15 Hz) and Beta (15-20 Hz) activity, but also discrete 2 Hz bands between 0-20 Hz were calculated.

Cognitive tests for assessing sleep loss

To evaluate the effects of sleep loss and how it may influence mGluR5 availability, subjects completed cognitive tests and validated questionnaires to examine subjective sleepiness (Karolinska Sleepiness Scale) (Åkerstedt and Gillberg, 1990), symptoms of tiredness (tiredness symptoms scale) (Bes et al., 1992), state and trait anxiety (State-Trait Anxiety Inventory) (Spielberger et al., 1970), affective state (Profile of Mood States) (McNair et al., 1971), and psychomotor performance (psychomotor vigilance test) (Durmer and Dinges, 2005).

PVT performance and the remaining questionnaires were assessed in 3-hour intervals throughout prolonged wakefulness, although two PVT sessions had to be abandoned due to PET image acquisition. To approach a normal distribution, mean reaction time (RT) on the PVT was expressed as speed (1/RT) and the number of lapses (RT > 500 ms) was transformed by ($\sqrt{x}+\sqrt{[x+1]}$).

Statistical analyses

All statistical analyses were performed with SAS® 9.1.3 software (SAS Institute, Cary, NC). To examine associations between mGluR5 availability and EEG markers of sleep need, Spearman rank correlation coefficients were calculated. To correct for multiple comparison when examining regional specific changes, we applied Bonferroni correction where the significance level was set to $\alpha < 0.0038$ (Bonferroni

correction: $\alpha = 0.05/13$). Furthermore we only considered effects significant when the alpha level for both baseline and sleep deprived conditions were achieved.

To assess effects of sleep deprivation related to the *FMR1* odd vs even genotype, mixed-effect models for analysis of variance (ANOVA) were calculated, including the factors 'genotype' (odd, even), 'condition' (sleep control, sleep deprivation) and 'session' (12-14 time points during prolonged wakefulness), as well as their interactions. Two-tailed, paired *t*-tests were conducted to localize significant differences. Significance level was set at $p < 0.05$.

To assess regional specific differences for the *FMR1* odd vs even genotype, the number of effective comparisons was reduced to the six regions previously shown to have increased mGluR5 availability following sleep deprivation (Hefti et al., 2013), As a result the significance level of the two-way ANOVAs were set to $\alpha < 0.00833$ (Bonferroni correction: $\alpha = 0.05/6$).

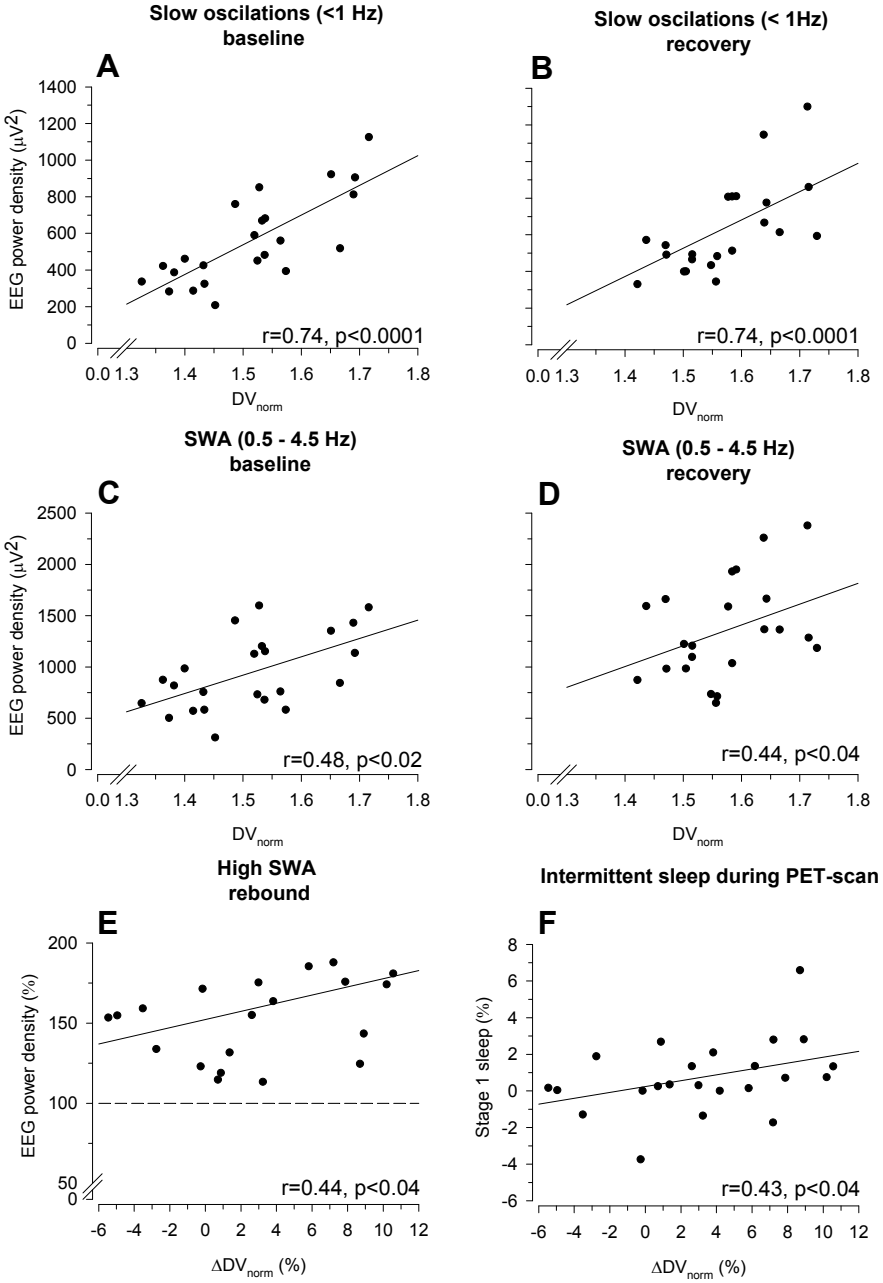
Results

EEG markers of sleep need is associated with mGluR5 availability

The strongest homeostatic markers for sleep need is EEG SWA (0.5 - 4.5 Hz), which is increased, especially in the first NREM sleep episode following sleep deprivation (Achermann and Borbély, 2011). On the other hand, slow-oscillations are a strong hallmark of NREM sleep and have been associated with the mGLuR5 (Blethyn et al., 2006) as well as learning and memory (Marshall et al., 2006). We previously showed how sleep deprivation leads to an increase in global mGluR5 availability, which positively correlates to increased subjective sleepiness (Hefti et al., 2013). Therefore, to further test the association between sleep need and global mGluR5 availability, we examined how EEG markers of sleep need in the first NREM sleep episode, is associated with global mGluR5 availability. EEG slow oscillations and SWA was calculated for baseline and recovery sleep and correlated with global mGluR5 availability after 9 or 33 hours of wakefulness respectively. A positive association was found for global mGluR5 availability with slow oscillations and SWA under baseline and sleep deprived conditions (Figure 1A – 1D). Also the spindle range (12 - 15 Hz) showed a trend-wise negative correlation with mGluR5 availability under baseline conditions ($r = -0.39$, $p < 0.07$) yet no other significant associations were found under neither condition. To examine the actual response to sleep deprivation, relative changes in global mGluR5 availability and EEG power was calculated. The increase in high SWA (2 - 4Hz) was significantly correlated with the increase in mGluR5 availability with sleep deprivation (Figure 1C), whereas no other frequency band revealed a significant association.

Finally we examined EEG markers of sleep and waking during the one hour PET scan. Although subjects were instructed to stay awake and were alerted via intercom if signs of sleep were detected, sleep deprivation reduced wakefulness (control: 62.2 ± 1.91 min; sleep deprived: 53.7 ± 2.97 min, $t = -3.74$, $p < 0.002$) and increased stage 1 (control: 2.07 ± 0.50 min; sleep deprived: 3.64 ± 0.77 min, $t = 1.87$, $p < 0.08$) and stage 2 (control: 2.90 ± 1.26 min; sleep deprived: 8.81 ± 1.94 min, $t = 4.15$, $p < 0.001$) sleep. The increase in intermittent stage 1 sleep significantly correlated with the increase in global mGluR5 availability after sleep deprivation ($r = 0.43$, $p < 0.04$) (Figure 1D).

Figure 1. Association between mGluR5 availability, EEG oscillations and markers of sleep need



To examine the association between mGluR5 and sleep need, objective markers of sleep loss was correlated with global mGluR5 availability in 26 healthy men. (**A – D)**: First NREM sleep episode for slow oscillations (<1 Hz) and SWA (0.5 - 4.5 Hz) for baseline and recovery conditions was positively correlated with global mGluR5 availability after 9 and 33 hours of wakefulness respectively. (**E)**: Relative change in first NREM sleep episode for high SWA (2-4 Hz) revealed a positive correlation with the relative change in global mGluR5 availability caused by sleep deprivation. (**F)**: Sleep deprivation induced changes in intermittent stage 1 sleep during the ~60 min PET-scan was positively correlated with changes in mGluR5 availability. Statistics calculated by Spearman's rank correlation coefficient.

To examine regional specific associations between mGluR5 availability and EEG markers of sleep need, we investigated 13 regions of interest. To correct for multiple

comparisons, regional correlations for baseline and the sleep deprived conditions were required to simultaneously survive Bonferroni correction with a significance level of $\alpha < 0.0038$. Out of the 13 regions investigated, slow oscillations were found to be positively correlated with regional mGluR5 availability in the parietal cortex, precuneus, striatum, anterior cingulate cortex, medial temporal lobe, frontal superior medial, orbitofrontal cortex and the dorsolateral prefrontal cortex (supplementary Figure 1, Appendix 3). On the other hand, SWA was found to be significantly correlated with mGluR5 availability in the parietal cortex (supplementary Figure 2, Appendix 3). None of the other 12 regions investigated remained significant following Bonferroni correction.

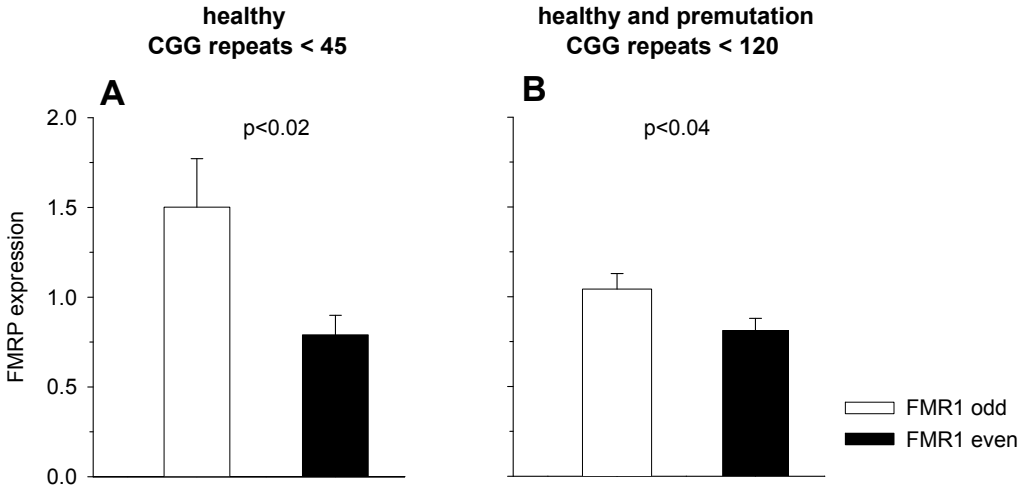
Odd vs. even numbers of CGG repeats modulate human FMRP expression

FMRP, which is transcribed by the *FMR1* gene, has been shown to play an important role in regulating neuronal plasticity. Although FMRP expression is reduced among FXS full mutation carriers (>200 CGG repeats) and negatively correlated with the number of CGG repeats among premutation (55-199 CGG repeats) carriers, this correlation does not persist in among healthy subjects (5-45 CGG repeats) (Peprah et al., 2009). Given how in vitro experiments have linked odd numbers of CGG repeats to more stable hairpins when compared to even (Darlow and Leach, 1998a), we hypothesized that *FMR1* CGG odd vs even repeats would affect RNA hairpin stability and thereby FMRP translation, although it might not directly modulate *FMR1* transcription levels. To examine this association we reanalyzed data from Allen et. al. 2004 and Peprah et al. 2009, who examined *FMR1* transcription and FMRP translation. Here we reanalyzed the expression levels measured in fresh human blood among healthy and FXS premutation carriers.

As previously described, CGG repeats in the healthy carrier range of 20-45, did not correlate with either *FMR1* levels ($r_p = 0.02$, $p > 0.94$; $r_s = -0.07$, $p > 0.78$; $n = 16$) or FMRP expression ($r_p = 0.01$, $p>0.96$; $r_s = 0.11$, $p > 0.65$; $n = 18$). Nevertheless, when comparing odd with even numbers of CGG repeats in the same range, *FMR1* mRNA levels were 32.2% increased (yet not significant) among *FMR1* odd, compared to even carriers (even: 1.04 ± 0.12, $n = 8$; odd: 1.38 ± 0.22, $n = 8$; two-tailed t-test: $p > 0.19$). FMRP expression on the other hand was significantly higher and revealed a 90.2% increase among *FMR1* odd carriers compared to even (Figure 2A). Analysis of both premutation and healthy carriers revealed similar *FMR1* levels (even: 1.72 ± 0.34, $n = 16$; odd: 1.63 ± 0.10, $n = 14$; two-tailed t-test: $p > 0.81$), but a significant increase in FMRP expression among odd compared to even carriers

(Figure 2B) corresponding to 28.4% increased FMRP expression for odd numbered CGG repeats.

Figure 2. FMRP expression levels among healthy and premutation FXS patients



Reanalysis of Peprah et. al. 2009 for comparison of odd vs. even number of *FMR1* CGG repeats among healthy (**A**) and premutation carriers (**B**). (**A)**: FMRP expression was increased by 90.2% among odd CGG carriers (n = 8) when compared to even (n = 10). (**B)**: For the entire sample of healthy and premutation carriers, FMRP expression was increased by 28.4 % when comparing odd (n = 36) to even (n = 41) CGG repeat carriers. Statistics are calculated by two-tailed student's t-tests and presented as means + SEM.

Odd vs. even number of *FMR1* CGG repeats modulate global mGluR5 availability

To further examine the FMRP/mGluR5 association model that suggest an inverse relationship between mGLuR5 and FMRP (Dölen et al., 2007; Dölen and Bear, 2008), we set out to examine the relationship between mGluR5 and FMRP under sleep control and sleep deprived conditions. All 26 subjects who underwent the study were genotyped for the number of CGG repeats in the *FMR1* gene. The number of CGG repeats was between 17 - 44, all in the healthy range (Maddalena et al., 2001). Subjects were grouped either as odd or even carriers, depending on their number of *FMR1* CGG repeats. The two groups were highly homogenous and did not differ with respect to age, body mass index, habitual sleep duration, habitual caffeine or alcohol consumption, anxiety, generalized sleepiness, sleep quality, Chronotype or verbal intelligence (table 1). Only habitual sleep duration revealed trend-wise sleep more among *FMR1* even carriers when compared to odd (table 1).

Table 1 Demographics among FMR1 odd vs even CGG repeat carriers

variable	*FMR1* odd	*FMR1* even	*p*
Group size (n)	12	14	-
Age (years)	23.8 ± 0.58	23.5 ± 0.58	0.752
Body mass index (kg/m^2)	22.8 ± 0.53	21.8 ± 0.51	0.211
Habitual sleep duration (hours)	7.19 ± 0.23	7.75 ± 0.17	**0.055**
Daily caffeine consumption (mg/day)	167 ± 39.2	138 ± 45.1	0.645
Alcohol consumption (drinks/week)	2.63 ± 0.46	4.21 ± 1.05	0.202
Trait Anxiety Inventory	36.4 ± 1.56	33.9 ± 2.30	0.397
Epworth sleepiness scale	6.50 ± 0.90	7.57 ± 0.92	0.418
Pittsburg sleep quality index score	4.25 ± 0.45	3.36 ± 0.49	0.195
MCTQ chronotype	4.30 ± 0.25	4.27 ± 0.21	0.856
MWT-B verbal IQ score	108 ± 3.42	104 ± 2.60	0.296

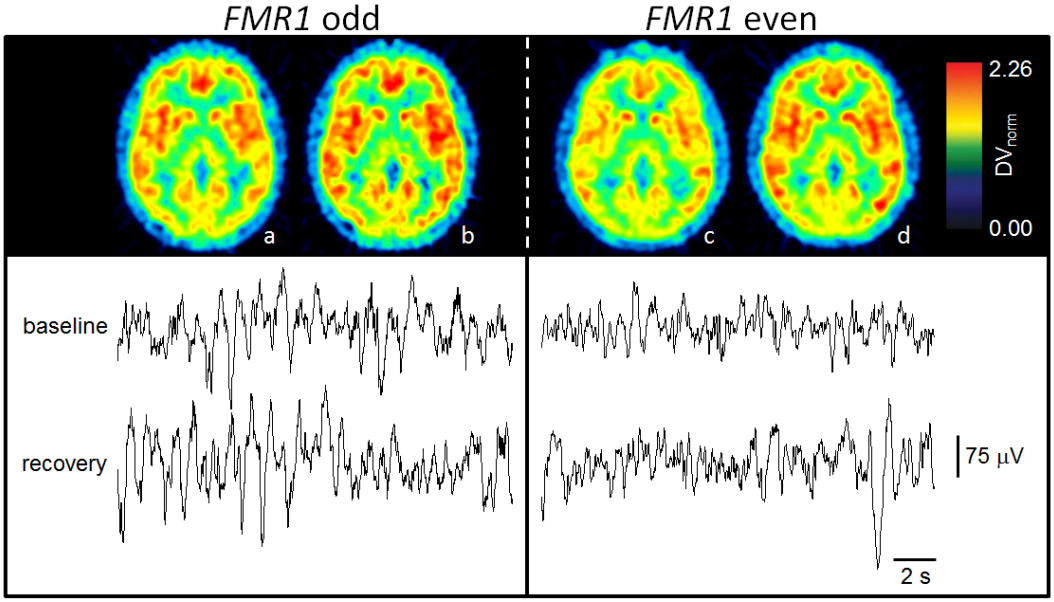
The demographic distribution of *FMR1* odd and even CGG repeat carriers were compared by validated questionnaires in German (Beck et al., 1961; Weyers et al., 1995; Francis et al., 2006; Viwatpinyo and Chongthammakun, 2009). With respect to age, BMI, habitual caffeine and alcohol consumption, trait anxiety, Epworth sleepiness scale, Pittsburg sleep quality score, chronotype and verbal IQ (Mehrfachwahl wortschatz intelligenz Test - B), the groups were undistinguishable. Only self-reported habitual sleep duration suggested an increased sleep need among *FMR1* even carriers compared to odd.

Effects of sleep deprivation on ^{11}C-ABP688 availability and EEG NREM sleep (exactly 30 min after sleep onset) for the odd and even *FMR1* group are illustrated in two representative individuals (Figure 3). The axial slice show parts of the anterior cingulate, insula, medial temporal lobe, medial superior frontal cortex, dorsolateral prefrontal cortex, striatum, precuneus, hippocampus and thalamus. Indeed, both sleep deprivation and *FMR1* odd vs. even genotype modulated global mGluR5 availability ('condition': $F_{1,21} = 10.79$, $p < 0.004$; 'genotype': $F_{1,21} = 1.93$, $p > 0.179$; 'condition x genotype' $F_{1,21} = 7.74$, $p < 0.02$). Post-hoc testing revealed higher levels of mGluR5 among *FMR1* odd carriers compared to even; under sleep control conditions (Figure 4A) whereas the availability under sleep deprived conditions were similar between the groups (even: 1.55 ± 0.03, odd: 1.57 ± 0.03, two-tailed t-test: $p > 0.60$). Despite *FMR1* odd and even carriers both displayed a positive mean increase in global mGluR5 availability after sleep deprivation, the increase was only significant among *FMR1* even carriers (*FMR1* even: $t = 4.14$, $p < 0.002$; *FMR1* odd: $t = 0.37$, $p = 0.718$; two-tailed paired t-tests) who also displayed a markedly greater rebound (Figure 4E).

Finally we investigated regional specific associations between mGluR5 availability and *FMR1* CGG odd vs. even genotype following sleep deprivation. Following Bonferroni correction, the main effect of sleep deprivation remained significant for the parrahippocampal gyrus, anterior cingulate, amygdala, medial temporal lobe and insula ('conditon': $F_{1,21} \geq 9.05$, $p < 0.007$). Although no main effect of genotype was

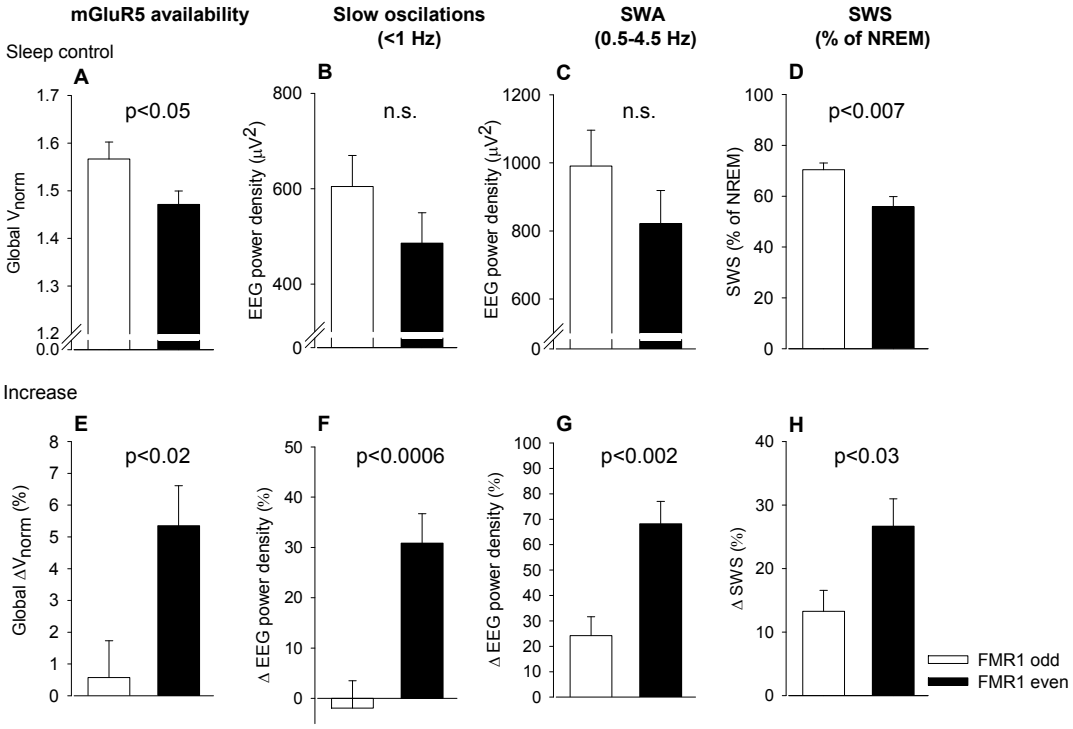
observed, the rebound following sleep deprivation was found to be significant for the medial temporal lobe and Amygdala ('condition x genotype' $F_{1,21}$ = 8.54, p < 0.0082). Similar as for the reported global changes in mGluR5 availability, the two significant regions revealed a highly elevated rebound among even *FMR1* CGG carriers when compared to odd, following sleep deprivation (data not shown).

Figure 3. Illustration of mGluR5 availability and NREM EEG oscillations in one representative *FMR1* odd and one *FMR1* even subject.



Top: Axial slice in two representative *FMR1* odd and even individuals, illustrating the binding of the non-competitive and highly selective mGluR5 antagonist ^{11}C-ABP688 in sleep control (~ 9 hours of wakefulness) and sleep deprived (~ 33 hours of wakefulness) conditions. As illustrated, both sleep deprivation and *FMR1* odd vs. even genotype ('condition': $F_{1,21}$=10.79, p<0.004; 'genotype': $F_{1,21}$=1.93, p=0.179; 'condition x genotype' $F_{1,21}$=7.74, p<0.02) modulated global mGluR5 availability. The axial slice show parts of the anterior cingulate, insula, medial temporal lobe, medial superior frontal cortex, dorsolateral prefrontal cortex, striatum, precuneus, hippocampus and thalamus (z = 2 mm according to the Montreal Neurological Institute brain atlas). **Bottom:** 20 s EEG traces in the same two individuals exactly 30 min after sleep onset in baseline and recovery sleep. The EEG traces illustrates the proposed link between slow waves and mGluR5 availability (see Figure 1 for quantification). Furthermore, it illustrates the increased SWS among *FMR1* odd carriers in baseline, as well as the enhanced rebound among even *FMR1* carriers for EEG slow-oscillations and SWS following sleep deprivation (see Figure 4 for quantification).

Figure 4. Comparisons of mGluR5 availability, NREM EEG oscillations, and their relation to the FMR1 odd vs even genotype.



Comparison of odd (white bars) vs. even (black bars) *FMR1* CGG repeat carriers, with respect to global mGluR5 availability (**A** & **E**), EEG sleep slow oscillations (**B** & **F**), EEG SWA (**C** & **G**) and relative SWS (**D** & **H**) in the first NREM sleep episode for sleep control (upper panel) and the rebound following sleep deprivation (lower panel). (**A)**: Global mGluR5 was significantly higher under control conditions for *FMR1* odd carriers compared to even. (**B** – **D)**: The three sleep parameters showed a similar increased among odd *FMR1* CGG carriers under baseline condition, however only for relative SWS was the association significant. (**E)**: The rebound in mGluR5 availability following sleep deprivation on the other hand, was significantly stronger among *FMR1* CGG even carriers. (**F** – **H)**: The rebound for all three sleep parameters revealed a similar increase among even *FMR1* CGG carriers compared to odd in response to sleep deprivation. Figure is presented as mean ± SEM. Comparisons are calculated with students two-tailed t-tests following a significant 'condition x genotype' interaction in the two-way ANOVAs (see text). Relative values are used for post-hoc testing the sleep deprivation induced changes.

Objective markers of sleep need is regulated by *FMR1* CGG odd vs. even genotype

The homeostatic regulation of slow oscillations (<1 Hz) and SWA (0.5 - 4.5 Hz) reflects rhythmic global oscillations in neuronal activity, although the exact underlying mechanisms remain unknown. Since overexpression of FMRP in drosophila melanogaster was shown to reduce neuronal plasticity and affect overall sleep duration, we wanted to examine how the odd vs. even number of CGG repeats in the *FMR1* gene might affect EEG markers of sleep need in the first non-REM sleep episode. Analysis of EEG spectral power revealed a genotype independent increase in delta, theta and alpha activity (0.5 - 11.5 Hz: 'deprivation': $F_{1,24} \geq 4.78$, $p < 0.04$) as

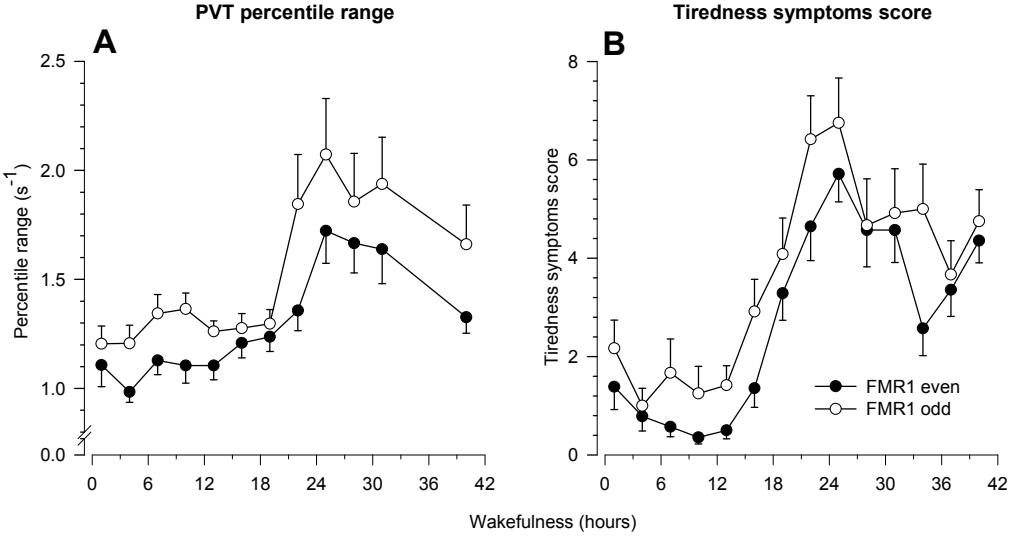
well as a significant reduction in low spindle activity (12.5 - 13.25 Hz: 'deprivation' $F_{1,24} \geq 9.90$, $p < 0.005$). A general increase among *FMR1* even carriers compared to odd was observed in theta and low alpha activity (7 - 10 Hz: 'genotype' $F_{1,24} \geq 4.54$, $p < 0.05$). Finally we observed a significant increase in delta and low theta activity (0.25 - 5.25 Hz: 'condition x genotype' $F_{1,24} \geq 4.68$, $p < 0.05$) as well as a reduction in spindle activity (13.5 - 13.75 Hz: 'condition x genotype' $F_{1,24} \geq 4.46$, $p < 0.05$) among *FMR1* even carriers when compared to odd, in response to sleep deprivation. To further examine the genotype dependent modulation in the slow wave range, we calculated total EEG power for slow oscillations and SWA as well as the relative amount of slow wave sleep (SWS) for the first NREM episode (Figure 4). ANOVA revealed significant modulations of condition and the genotype interaction, for all three sleep parameters ('condition': $F_{1,24} \geq 6.04$, $p < 0.03$; 'condition x genotype' $F_{1,24} \geq 5.87$, $p < 0.03$). A significant main effect of genotype was only found for relative SWS ('genotype' $F_{1,24} \geq 5.88$, $p < 0.03$). Comparison of the baseline condition revealed a nominal increase for all three-sleep parameters among the *FMR1* CGG odd compared to even carriers, however the difference was only significant for relative SWS (Figure 4B - 4D). No differences were observed for any parameter under sleep deprived conditions (data not shown). Finally, when analyzing the rebound for slow oscillations, SWA and SWS, all three parameters were significantly reduced among *FMR1* CGG odd carriers when compared to even (Figure 4F – 4H).

Objective and subjective measures of sleep need is higher among *FMR1* odd carriers compared to even

To quantify how sleep deprivation affects *FMR1* odd and even carriers throughout prolonged wakefulness, performance measures and ratings of sleepiness were examined in 3-hour intervals throughout sustained waking. Interpercentile performance (10-90th percentile) on the psychomotor vigilance task (PVT) a measure of consistent performance, is highly sensitive in detecting early sings of increased sleep pressure (Graw et al., 2004). Subjective signs of sleep need, can be examined by the tiredness symptoms scale (TSS) and the Karolinska sleepiness scale (KSS). Where the TSS is a 14-items checklist with physical and mental symptoms of tiredness (Bes et al., 1992), the KSS is a more subjective 9-point scale assessing increasing sleepiness. The interpercentile PVT performance revealed both a circadian and homeostatic component, with a strong main effect of time awake ('session' $F_{11,206} = 7.39$, $p < 0.0001$, Figure 5A). Independent of time awake, *FMR1* odd carriers demonstrated an overall reduced performance when compared to even ('genotype' $F_{1,40} = 4.87$, $p < 0.04$). Also KSS and TSS ratings revealed a strong

homeostatic and circadian modulation (KSS: 'session' $F_{13,192}$ = 17.2, p < 0.001; TSS: 'session' $F_{13,219}$ = 10.4, p < 0.0001). However like for interpercentile PVT performance, *FMR1* odd carriers demonstrated an overall increase in symptoms of tiredness when compared to even ('genotype' $F_{1,46.5}$ = 4.51, p<0.04, 'genotype x session' $F_{13,219}$ = 1.01, p > 0.44) (Figure 5B), although no association between KSS score and genotype was found ('genotype' $F_{1,55.1}$=1.10, p=0.30; 'genotype x session' $F_{13,192}$=1.21, p=0.27); (data not shown).

Figure 5. Psychomotor performance and signs of sleepiness during prolonged wakefulness, depends on the *FMR1* odd vs even genotype.



To quantify the effects of sleep deprivation, interpercentile PVT performance (10-90th percentile) a sensitive method for detecting sings of increased sleep pressure (Graw et al., 2004), and symptoms of tiredness (TSS) were assessed in 3-hour intervals throughout prolonged wakefulness. *FMR1* odd CGG repeat carriers showed reduced PVT performance ('genotype' $F_{1,40}$=4.87, p<0.04) (**A**) and increased symptoms of tiredness ('genotype' $F_{1,46.5}$=4.51, p<0.04) (**B**) throughout prolonged wakefulness when compared to even.

Discussion

This multimodal PET imaging study reveals for the first time a molecular marker for EEG slow oscillations (<1 Hz) and SWA (0.5 - 4.5 Hz) in humans in vivo, visible under both control and sleep deprived conditions. Slow oscillations during sleep are characterized by sequences of membrane depolarization and frequent neuronal firing (UP states) followed by membrane hyperpolarization and neuronal silence (DOWN states), typically at frequencies around 0.2 – 0.9 Hz (Steriade et al., 1993a; Achermann and Borbély, 1997). The slow oscillations are believed to primarily be cortically generated since they persists following thalamectomy (Steriade et al., 1993b) and are strongly reduced when cortical inputs are removed (Timofeev and Steriade, 1996). Nevertheless spike burst of thalamocortical neurons often proceeds cortical UP states in vivo suggesting that they might be importantly involved in the synchronization of slow oscillations, similarly as for spindles and slow waves (Steriade et al., 1993b; Contreras and Steriade, 1995). The slow oscillations are enhanced following NMDAR blockage, observed following ketamine administration (Feinberg and Campbell, 1993), but also following mGluR type 1 agonists in vitro (Hughes et al., 2002). Given how mGluR type 1 receptors are known to regulate NMDAR (Oliet et al., 1997; Collingridge et al., 2004), it is likely that mGluR5 play an important role for sleep slow oscillations and that increased availability of mGluR5 is associated with enhanced slow oscillations. Indeed, we here show a strong and highly significant positive association between global mGluR5 availability and slow oscillations under both baseline and sleep deprived conditions suggesting that mGluR5 may play an essential role in maintaining slow oscillations especially during the first NREM sleep episode. Indeed, enhancing slow oscillations in the early (but not late) part of NREM sleep has previously been shown to improve hippocampal dependent declarative memory retention in humans (Marshall et al., 2006), an effect which therefore could be linked to increased mGluR5 activation. Slow oscillations have previously been associated with specific increases in cerebral blood flow in the precuneus, posterior cingulate, medial frontal, parietal, and central gyrus (Dang-Vu et al., 2008), brain regions which are remarkably similar to where we find the strongest association between mGluR5 availability and slow oscillation power. Indeed the mGluR5 availability in the parietal cortex and the precuneus explain between 64% and 70% of the variance in slow oscillation power under both sleep control and sleep deprived conditions. Also SWA, a strong marker of homeostatic sleep need was found to be significantly associated with global availability of mGluR5 under both sleep control and sleep deprived conditions. Also the relative increase in high SWA

(2 – 4 Hz) was positively associated with the increase in global mGluR5. This shows that mGluR5 does not only play a role for EEG slow oscillations but is also involved in the regulation of homeostatic sleep need. Homer 1a was previously shown to be a core molecular marker of sleep need in the mouse (Maret et al., 2007) but in humans, no such molecular marker has previously been described. Homer1a is known to selectively uncouple mGluR5 from effector targets in the membrane of the postsynaptic density and attenuates the mGluR5-mediated rise in intracellular calcium levels (Kammermeier and Worley, 2007). This interaction between Homer1a and mGluR5 is necessary for mGluR5-dependent synaptic LTD (Ronesi and Huber, 2008).

In the second part we analyzed genetic influences of the trinucleotide CGG repeat in the *FMR1* gene to investigate the role of FMRP on mGluR5 availability and sleep need. Although the expansion of the trinucleotide CGG is known to cause FXS and the complete loss of FMRP (Cummings and Zoghbi, 2000), CGG's in the healthy range (< 45) had newer previously been shown to modulate FMRP expression, although RNA stemloops had been suggested to affect expression in vitro (Darlow and Leach, 1995; 1998a; 1998b). By reanalyzing previously published data on *FMR1* and FMRP expression levels (Allen et al., 2004; Peprah et al., 2009), we here show how stemloops may influence FMRP expression by revealing an almost two-fold increase in FRMP among healthy carriers of odd number *FMR1* CGG's when compared to healthy even carriers. This higher FMRP expression associated with odd numbered CGG's persisted even when examining the entire sample of healthy and premutation carriers where an increase in FMRP expression of almost 30% was found to be associated with the odd number of CGG's compared to even.

Finally, based on the odd or even number of *FMR1* CGG repeats, we examined how FMRP expression may influence mGluR5 availability and sleep need. The mGluR5 and FMRP have been shown to have opposing roles, were activation of mGluR5 was associated with increased mRNA translation, FMRP would suppress it (Dölen et al., 2007; Dölen and Bear, 2008). Inspection of Drosophila melanogaster mutants revealed that FMRP expression is inversely linked to sleep duration (Bushey et al., 2009), and that overexpression of FMRP leads to reduced neuronal plasticity in response to sleep deprivation (Bushey et al., 2011). Indeed, we here show how the allegedly overexpressing odd CGG carriers report to sleep 30 min less every night when compared to even CGG carriers. Although this effect was only trend-wise significant, it further supports the functional significance of the odd/even *FMR1* CGG

repeat polymorphism. Fascinatingly, odd *FMR1* CGG repeat carriers were found to have increased global mGluR5 availability under control conditions when compared to even, a difference corresponding to 6.5% or almost twice the difference as observed overall following sleep deprivation. When analyzing differences in baseline sleep parameters in the first NREM episode, odd *FMR1* CGG carriers had a larger percentage of SWS when compared to even, corresponding to more than 15 minutes. Nevertheless, the overall length of the NREM episode was not different between the two genotypes. This increase in EEG markers of sleep need among *FMR1* CGG odd carriers was also observed for EEG slow oscillations and SWA although the difference did not reach significance. This difference in global mGluR5 availability at control conditions and the increased SWS in baseline were unexpected, especially since the odd *FMR1* CGG carriers reported reduced habitual sleep. Therefore, these findings may suggest a faster dissipation of sleep pressure among the odd CGG *FMR1* carriers, or it may suggest a compensatory mechanism due to chronically increased FMRP expression. An answer may come from the rating of sleepiness and PVT performance during prolonged wakefulness, where odd CGG *FMR1* carriers were found to have a chronically poorer PVT performance and increased signs of sleepiness. Taken together, this would suggest that the baseline differences in mGluR5 availability and SWS are related to compensatory mechanism due to chronically increased FMRP.

Finally we observed a highly blunted response to sleep deprivation among the odd *FMR1* CGG repeat carriers compared to even, observed for global mGluR5 availability, EEG slow oscillations, SWA and SWS. These findings appear to confirm what has been observed among FMRP overexpressing flies, which show signs of reduced neuronal plasticity following sleep deprivation (Bushey et al., 2011) suggesting that odd CGG *FMR1* carriers are especially vulnerable to sleep deprivation.

Taken together, our data reveal an important role for the mGluR5 in regulating EEG markers of sleep need and especially for the <1 Hz slow oscillations. Furthermore, mGluR5 may be considered a molecular marker of sleep need in humans in vivo. On the other hand, odd or even numbers of CGG's in the *FMR1* gene may affect FMRP expression even among healthy individuals. Odd CGG carriers with increased FMRP expression reveal increased mGluR5 availability and SWS under normal conditions, yet a strongly reduced response in mGluR5

availability and markers of sleep need following sleep deprivation, effects that further corroborates findings in FMRP overexpression flies.

Acknowledgments

This work was supported by Swiss National Science Foundation Grant #320030-135414, National Center of Competence in Research Neural Plasticity and Repair, and Novartis Foundation for Biomedical Research. ABP688 and its precursor were provided by Novartis Pharma AG.

We cordially thank Drs. Allen, Peprah and Sherman from the department of human genetics, Emory University, Atlanta, USA, for providing us with their data on FMR1 transcript and FMRP translation levels. Furthermore we thank all members of the Section of Chronobiology and Sleep Research, Institute of Pharmacology and Toxicology, University of Zürich, and the Division of Nuclear Medicine, University Hospital Zürich, who helped with data collection and analyses.

Drs. Sovago and Gomez-Mancilla are employees of Novartis Pharma AG (Basel), which is developing and testing drugs targeting the metabotropic glutamate receptor subtype 5. Mr. Holst, Dr. Hefti, Dr. Buck, Dr. Ametamey, Mr. Scheidegger, Dr. Dürr Dr. Baumer, Dr. Seifritz, and Dr. Landolt report no biomedical financial interests or potential conflicts of interests.

Chapter 6

Concluding remarks

By using an integrative approach, this thesis investigated molecular aspects of sleep-wake regulation in humans. Roles for the dopaminergic and glutamatergic systems were examined, by studying genetic variants, molecular brain imaging, pharmacological interventions, polygraphic EEG recordings, actigraphy and cognitive performance in healthy human volunteers.

In the first two studies, genetic polymorphisms of the genes coding for the dopamine metabolizing enzyme catechol-O-methyl transferase (COMT) and for the dopamine transporter (DAT), were determined in 129 healthy adult subjects who underwent continuous rest-activity monitoring for roughly 4 weeks. Following the actigraphy study, effects related to the DAT polymorphism on sleep-wake regulation were investigated in 57 volunteers in a controlled sleep deprivation study. The protocol consisted of a baseline sleep opportunity and 40 hours of sustained wakefulness, followed by a recovery sleep episode. Furthermore, subgroups of these participants completed the study protocol twice, where 2 x 200 mg caffeine (n=16) or 2 x 100 mg modafinil (n=22) were administered in double-blind, placebo-controlled manner, during prolonged waking. The response to sleep deprivation and to the two treatments, were assessed by analyses of EEG spectra, individual slow wave characteristics and questionnaires.

In the second two studies, positron emission tomography (PET) imaging was used to investigate the availability of metabotropic glutamate receptors of subtype 5 (mGluR5) in the human brain. The highly selective, non-competitive radioligand ^{11}C-ABP688 was used for mGluR5 quantification. In total, 26 healthy men underwent a two-week, randomized, crossover study protocol where the PET scans were scheduled after either 9 or 33 hour of sustained wakefulness.

An overview of the results in this thesis is summarized in Table 1. In the following sections, the most important results are presented in a broader context and related to current research.

DAT dependent dopaminergic neurotransmission modulates sleep-wake regulation

As described in the introduction (Chapter 1), the role for dopamine (DA) in modulating sleep and wakefulness was long thought to be of minor importance because of inconsistent alterations of DA across the sleep-wake cycle in cats (Miller et al., 1983; Steinfels et al., 1983; Trulson and Preussler, 1984). However, the prevailing opinion changed, when it could be shown that: enhanced dopaminergic transmission induced wakefulness in mice and Drosophila (Wisor et al., 2001; Kume et al., 2005); that the genetic ablation of DA D_2 receptors in mice was associated with prolonged sleep time (Qu et al., 2010); and that the firing rate of dopaminergic neurons changed across the sleep wake cycle in rats (Léna et al., 2005; Dahan et al., 2007). Altered dopaminergic neurotransmission also interferes with sleep and wakefulness in humans. The loss of dopaminergic cells in the substantia nigra (SN) observed in Parkinson's patients, not only causes motor symptoms, but is also associated with disturbed sleep (Lees et al., 1988) and increased nocturnal activity as demonstrated by continuous activity monitoring (van Hilten et al., 1994). Similarly, patients diagnosed with attention deficit hyperactive disorder (ADHD) show reduced nocturnal sleep efficiency, which can be improved by methylphenidate (Sobanski et al., 2008), a treatment which, at least in part, inhibits DAT and thereby increases dopaminergic neurotransmission (Volkow et al., 1998).

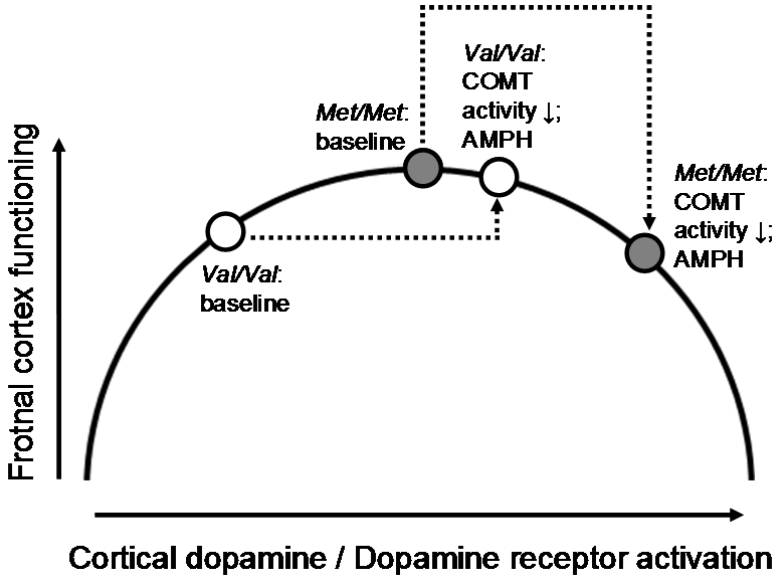
In Chapters 2 and 3, it was investigated whether the dopaminergic system affects long-term habitual sleep-wake patterns, effects of acute sleep loss on markers of sleep homeostasis, and interactions of homeostatic sleep regulation with caffeine and modafinil. To address these questions, distinct polymorphisms that have been reported to affect dopaminergic neurotransmission were investigated. Specifically, the *COMT* Val158Met polymorphism (SNP-ID: rs4680) and the 3'-untranslated region (UTR) variable-number-tandem-repeat (VNTR) polymorphism of *DAT1* (SNP-ID: rs28363170) were investigated. The polymorphism of COMT is associated with a Val-to-Met amino acid substitution in the COMT protein, which decreases enzymatic activity in Met-allele carriers (Akil et al., 2003; Chen et al., 2004). Furthermore, the *DAT1* polymorphism has been associated with increased striatal DAT availability in 9-repeat allele carriers (9R) compared to 10-repeat allele homozygotes (10R/10R) (van de Giessen et al., 2009; Costa et al., 2011; Spencer et al., 2013).

Interestingly, the DAT and COMT proteins show different regional distributions in human brain. The DAT is primarily expressed in the striatum and thought to be the rate limiting protein, responsible for striatal dopaminergic neurotransmission (Ciliax et al., 1995; 1999; Lewis et al., 2001). On the other hand, COMT is mainly expressed in the prefrontal cortex and only to a lesser extent in the striatum (Meyer-Lindenberg et al., 2005; Tunbridge et al., 2006). Such expression patterns cannot completely be separated, given how altered striatal dopaminergic neurotransmission have been reported to modulate prefrontal dopamine transmission (Kandel et al., 2000). Nevertheless, the simultaneous examination of these two individual polymorphisms, not only enabled an investigation of the impacts of the dopaminergic system on sleep-wake regulation, but also to some extent, to discriminate between effects of prefrontal and striatal DA neurotransmission. Investigation of long-term habitual sleep-wake regulation (Chapter 2) was performed by a wrist-actimetry device as previously validated for sleep-wake estimates (Sadeh, 2011). Subjective questionnaires, as well as activity over roughly 4-weeks, with no behavioral restrictions, were analyzed for variables including total daily activity, as well as the most active 10-hours and least active 5-hours (L5). The polymorphism of COMT was found to modulate BMI and the change in sleep duration from weekdays to rest days. On the other hand, the *DAT1* polymorphism was associated with differences in subjective sleepiness and daily rest-activity patterns in men. Here, the *DAT1* 9R carriers revealed increased prevalence of elevated daytime sleepiness, increased overall motor activity and an augmented activity during L5 when compared to 10R/10R. The data suggest that mainly the polymorphism *of DAT1*, but to some extent also the *COMT* polymorphism, may modulate sleep-wake regulation.

Given the limitations of actigraphy to unambiguously quantify wakefulness and sleep (Sadeh, 2011) and the lack of a challenge to the sleep-wake regulatory systems, caution should be taken in interpreting these findings. Therefore, to investigate associations of the *DAT1* polymorphism and sleep-wake regulation, the effects of sleep deprivation were examined in 57 genotyped individuals of previous laboratory studies. For COMT, such studies have been performed previously, and revealed no impact of the *COMT* polymorphism on homeostatically regulated markers of sleep need (Bodenmann et al., 2009a; 2009b; Bodenmann and Landolt, 2010). However, it should be noted that previous studies by Bodenmann and colleagues, focused solely on homozygote carriers of *COMT* Val158Met genotypes. The effects of the *COMT* polymorphism reported in Chapter 2, highlight a difference between homozygote and heterozygote Val158Met carriers. The findings are consistent with

the proposed inverted-U-shape relationship relating prefrontal cortical functions with dopaminergic neurotransmission (Figure 1) (Tunbridge et al., 2006). Consequently, future studies, including interventions, are warranted to examine the effects of COMT in relation to sleep homeostasis, not only to investigate the difference between homo- and heterozygotes, but also to investigate causality.

Figure 1: Modulations of frontal cortex functions by catechol-*O*-methyl transferase



Frontal cortex function is hypothesized to be modulated by dopamine in an inverted-U-shaped manner, which depends on the activity of COMT At baseline, high COMT activity in Val/Val homozygous subjects may be associated with suboptimal pre-frontal cortex (PFC) dopamine levels/function, whereas homozygous Met/Met allele carriers may have near-optimal PFC dopamine levels/function. By increasing cortical dopamine, for instance by amphetamine (AMPH) administration, Val/Val homozygous subjects can be pushed nearer to the peak of the inverted-U-shaped curve and improved frontal functioning. On the other hand, increasing dopaminergic tone by AMPH may not change or even decrease PFC functioning in COMT Met/Met homozygotes. Adapted from Tunbridge et al., 2006.

One way to address causality is to pharmacologically inhibit COMT, which can be achieved by administration of Tolcapone, a COMT inhibitor used for secondary treatment of Parkinson's disease (Dingemanse et al., 1995). Similarly, to study the role of DAT, blockers such as cocaine (Kilty et al., 1991) or modafinil (Volkow et al., 2009a) may be used. Indeed, modafinil has been suggested to work, at least in part, by binding to and inhibiting DAT (Mignot et al., 1994; Madras et al., 2006; Minzenberg and Carter, 2008; Volkow et al., 2009a). Thus, administration of modafinil during sleep-deprivation may provide valuable insights into the involvement of DAT on sleep homeostasis.

In Chapter 3, we examined effects related to the *DAT1* polymorphism on homeostatic EEG markers of sleep need during a strictly controlled sleep deprivation study. Both, 9R-carriers and 10R/10R genotypes of *DAT1* revealed a normal response to sleep deprivation (i.e. enhanced slow wave sleep (SWS), slow wave activity (SWA), and number of slow waves). Nevertheless, the rebound was modulated by *DAT1* and attenuated among 10R/10R carriers when compared to 9R. The effects of *DAT1* on SWS corresponded roughly to a 15 min larger rebound following sleep deprivation in 10R/10R compared to 9R-carriers. The 10R/10R genotypes exhibited on average an increase in SWS of ~64 minutes after sleep deprivation (Appendix 1). Accordingly, the effect of the *DAT1* polymorphism may account for ~23% of the difference in SWS rebound after sleep deprivation. As reported elsewhere, the polymorphism of *DAT1* has been linked to an approximately 15-20 % reduced striatal DAT availability in 10R/10R compared to 9R-carriers (Jacobsen et al., 2000; van Dyck et al., 2005; Youdim et al., 2006; van de Giessen et al., 2009; Costa et al., 2011; Spencer et al., 2013), which appears to correspond well to the findings reported in Chapter 3. However, DA modulates G-protein coupled receptors, which are known to provide a powerful mechanism of signal amplification (Nelson and Cox, 2008) and it therefore may not be meaningful to directly compare effect sizes of SWS and DAT expression. Nevertheless, the data support preclinical evidence for a role of DAT in modulating sleep and waking (Greenspan et al., 2001; Wisor et al., 2001; Kume et al., 2005) and the notion that the striatum and nucleus accumbens (NAc) may play an important role in maintaining wakefulness (Lazarus et al., 2012; 2013).

In the striatum, stimulatory (G_s-coupled) A_{2A} receptors are preferentially found post-synaptically on dendritic spines of GABAergic medium spiny neurons, where they form heteromers with inhibitory dopamine (G_i-coupled) D_2 receptors and (G_q-coupled) mGluR5 (Ferré et al., 2002; Ciruela et al., 2006; Ferré et al., 2007). Under normal conditions (Figure 2A) the concentrations of glutamate and adenosine are low, which favors activation of DA D_2 receptors. Lazarus and colleagues suggested that activation of DA D_2 receptors in the striatum leads to activation of the ascending reticular arousal system (ARAS), which promotes wakefulness (Chapter 1, Figure 6) (Lazarus et al., 2012). On the other hand, stimulation of mGluR5 in mGluR5-A_{2A}-D_2 heteromers, causes reduced affinity of DA for D_2 receptors, whereas co-stimulation of both A_{2A} and mGluR5 reduces the affinity of DA for D_2 receptors even further (Popoli et al., 2001). This in turn reduces ARAS activation and favors sleep (Figure 2B). Additionally, A_{2A} receptor antagonists such as caffeine will enhance

wakefulness. Furthermore, also modafinil, by enhancing dopamine neurotransmission, will promote wakefulness by increasing the activation of DA D_2 receptors (Figure 2).

It is well established that caffeine attenuates EEG SWA (Landolt et al., 2004), and that the interference is modulated by the adenosine A_{2A} receptor ADORA2A polymorphisms (Rétey et al., 2007; Bodenmann et al., 2012). In Chapter 3, the stimulating effects of caffeine and modafinil were investigated with respect to the *DAT1* polymorphism. The results revealed that the response and sensitivity is modulated by the *DAT1* polymorphisms, suggesting a striatial dopaminergic modulation of the stimulant effects of modafinil and caffeine. The findings are consistent with the notion that DAT knock-out mice are hypersensitive to caffeine, yet unresponsive to modafinil (Wisor et al., 2001).

Figure 2. Striatal receptor heteromers interconnects metabotropic glutamate receptors of subtype 5 (mGluR5), adenosine A_{2A} receptors and dopamine D_2 receptors.

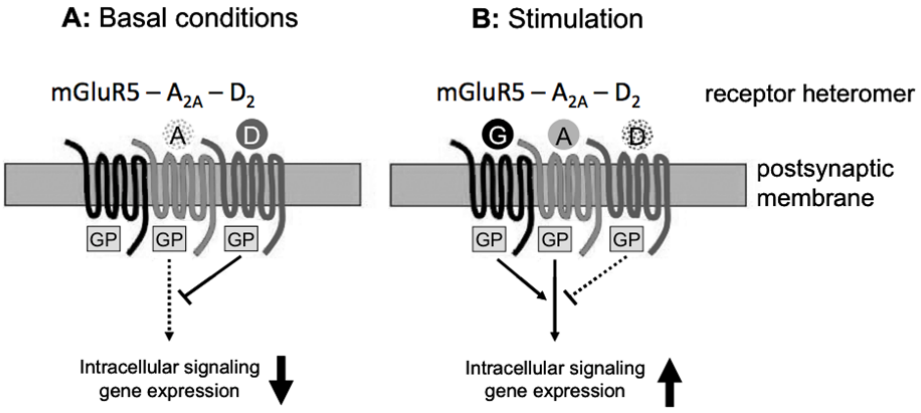


Illustration of mGluR5-A_{2A}-D_2 striatal receptor heteromers, which are mainly expressed postsynaptically on the dendritic spines of GABAergic medium spiny neurons. **A:** Under basal conditions, striatal glutamate and adenosine concentrations can be considered low. This favors D_2 receptor signaling which in turn leads to the activation of the ARAS and thereby enhanced wakefulness. **B:** Following prolonged wakefulness, glutamate and adenosine concentrations are hypothesized to increase and bind to their respective receptors in mGluR5-A_{2A}-D_2 receptor heteromers. This results in a decreased affinity of D_2 receptors for dopamine. This in turn enhances NAc inhibition on the ARAS and thereby promotes sleep.
mGluR5: metabotropic glutamate receptor of subtype 5, A_{2A}: adenosine A_{2A} receptor, D_2: dopamine D_2 receptor, G: glutamate, A: adenosine, D: dopamine, GP: G-protein. Adapted from Ferré et al., 2007; Bodenmann, 2009.

Glutamatergic mGluR5 dependent neurotransmission modulates sleep wake regulation

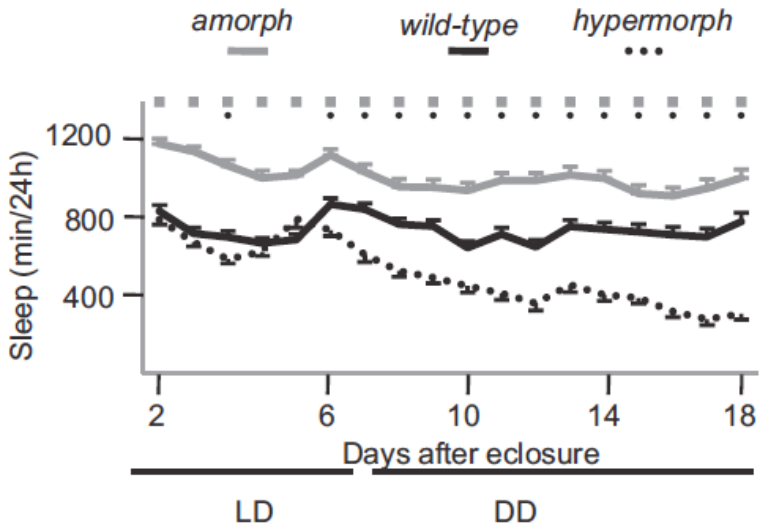
Through the heteromeric interaction with dopamine D_2 and adenosine A_{2A} receptors in the striatum described above, activation of mGluR5 may promote sleep.

The mGluR5 are mainly expressed on postsynaptic membranes of neurons and astrocytes in corticolimbic areas of the brain. These include the medial-prefrontal and orbitofrontal cortex, cingulate, striatum, amygdala, and hippocampus (Ametamey et al., 2007; Gasparini et al., 2008). Given the central role of glutamate in the human brain, it may not be surprising that the mGluR5 have been proposed to modulate a number of brain processes, including anxiety, memory, autism, learning, pain perception, depression and addiction (Swanson et al., 2005; Barker et al., 2006; Dölen et al., 2007; Nelson and Cox, 2008; Xu et al., 2009; Deschwanden et al., 2011; Jung et al., 2011; Hulka et al., 2013). Indeed, mGluR5 are known to modulate synaptic plasticity, long-term depression (LTD) and long-term potentiation (LTP) (Lu et al., 1997; Anwyl, 2009; Ayala et al., 2009; Izumi and Zorumski, 2012), which may explain why mGluR5 play a role in such a wide range of brain processes. However, the hypothesis that mGluR5 play a specific role in sleep-wake regulation is not only based on the striatal heteromeric interaction between mGluR5, DA D_2 and adensosine A_{2A} receptors. The mGluR5 also interact with the immediate early gene Homer1a, a proposed molecular marker of sleep homeostasis (Maret et al., 2007), which attenuates the mGluR5-mediated rise in intracellular calcium (Kammermeier and Worley, 2007). Homer1a-mGluR5 interaction is required for mGluR5-dependent synaptic LTD (Ronesi and Huber, 2008). Another important possible link between sleep-wake regulation and mGluR5, is its interaction with brain-derived neurotrophic factor (BDNF). The pharmacological activation of mGluR5, induce BDNF expression in cortical neurons and glia cells in rats (Legutko et al., 2006; Viwatpinyo and Chongthammakun, 2009). The BDNF expression in the cerebral cortex is high during wakefulness, low during sleep, and increased after sleep deprivation (Cirelli and Tononi, 2000). Furthermore, cortical injection of BDNF to awake animals promotes synaptic strength and enhances SWA in subsequent NREM sleep (Faraguna et al., 2008). Finally, mGluR5 has been proposed to be tightly linked with the Fragile X mental retardation protein (FMRP) (Dölen et al., 2007). Although the roles for FMRP in human sleep have previously not been explored, FMRP levels in Drosophila are inversely linked to sleep duration (Bushey et al., 2009) (Figure 3), and overexpression of FMRP in the fly model results in reduced neuronal plasticity in response to sleep deprivation (Bushey et al., 2011).

In human volunteers, sleep deprivation increased cerebral mGluR5 availability by roughly 3.5%, whereas the increase in anterior cingulate cortex, insula, medial temporal lobe, parahippocampal gyrus, striatum, and amygdala withstood statistical correction for multiple comparisons (Chapter 4). Furthermore, the increase in global

and regional mGluR5 availability was correlated with the change in subjective sleepiness at the time of PET imaging.

Figure 3. The dFmr1 gene in drosophila mutants modulates sleep time.



Comparison of amorph, hypermorph, and wild type flies, showing reduced, enhanced, or normal FMRP expression, respectively. Sleep across 24 hours was tracked, first under light-dark (LD) and later dark-dark (DD) conditions. The figure illustrates that FMRP is inversely linked to sleep time, such that FMRP over-expression is associated with reduced sleep time and reduced FMRP expression associated with increased time asleep. Upper panel gray squares: significant differences between wild-type and amorph flies, upper panel black dots: significant difference between hypermorph and wild-type files. Figure from Bushey et al., 2009

In Chapter 5, it was further examined whether sleep deprivation-induced changes in mGluR5 are related to EEG oscillations in baseline and recovery sleep. Indeed, mGluR5 availability in sleep control and sleep deprivation conditions was correlated with EEG SWA (0.5 – 4.5 Hz) and EEG slow oscillations (< 1 Hz) in baseline and recovery sleep. Furthermore, the increase in mGluR5 was specifically correlated with the increase in high SWA (2 - 4 Hz). Together, these findings suggest a tight relationship between mGluR5 availability and sleep-wake regulation. To further examine if the link between mGluR5 and sleep need is really causal, pharmacological intervention would be required to either block or activate mGluR5. Such an intervention would make it possible to document causal impacts on sleep and wakefulness. Interestingly, selective mGluR5 positive allosteric modulators were shown to increase locomotor activity and indirectly reduce delta sleep (Parmentier-Batteur et al., 2013). Intriguingly, preliminary observations in rats suggest that mGluR5 receptor antagonists consolidate deep sleep and enhance sleep efficiency, whereas allosteric activation of mGluR5 increase waking and decrease deep sleep

(Ahnaou et al., 2012; Huysmans et al., 2012). These observations provide a causal link between mGluR5 and sleep wake regulation, but in an inverse way as hypothesized by the mGluR5-D_2-A_{2A} receptor heteromer theory (Lazarus et al., 2012; 2013). Although preclinical result from animals cannot always be directly compared to human data, this emphasises the complex nature of neurotransmitter interactions in modulating sleep and waking. Interpretation of the preliminary data reported by Ahnaou and Huysmans in combination with the results reported in chapter 4 and 5, may suggest that the build-up of mGluR5 observed following sleep deprivation, depicts a compensatory mechanisms, promoting wakefulness and allowing the subjects to stay awake. However, this interpretation assumes that glutamatergic neurotransmission is unaltered following sleep deprivation, and that the preliminary data from Ahnaou and Huysmans will endure peer-reviewing. Nevertheless, it further highlights the importance of establishing how endogenous glutamate concentration are affected by sleep deprivation, for instance by the use of magnetic resonance spectroscopy.

If the enhanced mGluR5 availability following sleep deprivation is really a compensatory mechanism to promote wakefulness, rather than an actual marker of sleep need, this may explain why the correlation between EEG SWA and global mGluR5 availability under baseline and sleep deprived conditions, although significant, explains “only” about 19-23% of total SWA variance. On the other hand, the correlation between global mGluR5 availability and EEG slow oscillations in baseline and recovery, explain almost 55% of the variance in EEG power in this frequency band, supporting the role for mGluR5 receptors in modulating these slow brain oscillations (Cobb et al., 2000; Hughes et al., 2002; Blethyn et al., 2006). Indeed, slow oscillations have been linked to neuronal plasticity and memory processing (Steriade and Timofeev, 2003; Marshall et al., 2006), processes hypothesized to benefit from sleep (Tononi and Cirelli, 2003; 2006; Diekelmann and Born, 2010; Rasch and Born, 2013).

Finally, the link between sleep-wake regulation, mGluR5 and FMRP was analyzed by examining effects of the *FMR1* CGG repeat polymorphism among the 26 healthy men who had mGluR5 availability quantified after 9 and 33 hour of wakefulness. As already mentioned, the role of FMRP in sleep-wake regulation was studied in amorph (reduced FMRP expression) and hypermorph (enhanced FMRP expression) dFmr1 Drosophila mutants (Bushey et al., 2009) (Figure 3). Whereas the dFmr1 amorphs were long sleepers, dFmr1 hypermorphs were short sleepers and the wild-type fly

revealed a intermediate sleep phenotype, suggesting a dose-dependent relationship between FMRP expression and sleep time (Bushey et al., 2009). In humans, the *FMR1* CCG repeat polymorphism is known to be the cause of Fragile X syndrome (FXS), where a repeat expansion of >200 CGGs leads to DNA hypermethylation, gene silencing, and complete loss of FMRP (Cummings and Zoghbi, 2000; Barker, 2002). In healthy CGG repeat carriers (5 - 45 CGG repeats) no association between FMRP expression and number of CGG repeats has previously been described (Peprah et al., 2009). Nevertheless, in vitro experiments have linked odd numbers of CGG repeats to more stable RNA hairpins when compared to even repeat numbers (Darlow and Leach, 1998a). This observation lead to the hypothesis that *FMR1* CGG odd vs. even repeats, would affect RNA hairpin stability and thereby FMRP translation. By reanalysing data from Peprah et al. (2009), we found that odd *FMR1* CGG repeat carriers have a roughly 90% higher FMRP expression when compared to even number repeat carriers. By investigating this odd vs. even *FMR1* CGG repeat polymorphism we observed that this genetic variant modulates not only mGluR5 availability and percentage of SWS under baseline conditions, but also the rebound caused by sleep deprivation for mGluR5 availability, EEG slow oscillations, EEG SWA and the percentage of SWS. Similarly to FMRP over-expressing Drosophila mutants (Bushey et al., 2011), the rebound in the neurophysiological markers of sleep homeostasis was blunted in the high FMRP expressing odd *FMR1* CGG repeat carriers (Chapter 5).

Perspectives

In conclusion, the results of this thesis enhance our understanding of the molecular bases of sleep-wake regulation in healthy humans. Thus, both the dopaminergic and glutamatergic neurotransmitter systems play important roles in sleep homeostasis. Furthermore, the integrative approach applied here, combining molecular imaging, electroencephalographic recordings, human genetics, and subjective questionnaires; is a powerful tool to investigate complex human traits such as homeostatic sleep-wake regulation. Specifically, this thesis shows that DAT and mGluR5 play a role in human sleep-wake regulation. This basic knowledge may be crucial for the development of future pharmacological treatments of disturbed wakefulness and sleep, as it unravels new aspects of pharmacogenetics, the response to stimulants, and previously unknown aspects of the mGluR5-FMRP interaction.

Table 1: Synopsis of the results in the current thesis

Topic	Subjective effects	Behavioural and electroencephalographic effects	Neuroimaging
Motor-activity behaviour modulated by *COMT* (Val158Met) and *DAT1* (9R-10R) polymorphisms **Chapter 2**	Effects of *DAT1* (9R vs. 10R/10R) polymorphism: • Increased likelihood of elevated daytime sleepiness among 9R carriers. DAT & COMT combined: • Similar chronotypes.	Motor activity for *DAT1* polymorphism: • Motor activity modulated by gender and DAT1 genotype. Genotype dependent effects only observed among males. • Overall increased motor activity among male 9R carriers. • L5 activity increased among male 9R carriers. Motor activity for *COMT* (Val158Met) polymorphism: • The increase in rest-activity from weekdays to weekends is attenuated among Val/Met heterozygotes • BMI is reduced among Val/Met heterozygotes DAT & COMT combined: • Similar activity patterns, no epistatic interactions	n/a
Acute sleep loss and the response to the stimulants caffeine and modafinil is modulated by the *DAT1* (9R-10R) polymorphism **Chapter 3**	Effects of *DAT1* (9R vs. 10R/10R) polymorphism: • Reduced caffeine sensitivity associated with the 9-allele.	EEG markers associated with *DAT1* (9R vs. 10R/10R) polymorphism: • Caffeine modulates beta activity during waking in DAT1 genotype dependent manner. • Enhanced caffeine sensitivity among 10R/10R carriers for the number, amplitude, and slope of slow waves (< 2 Hz). • Enhanced response to modafinil among 9R-carriers for sigma activity during sleep. • “Normal” response to sleep deprivation for both DAT1 genotypes.	n/a

Topic	Subjective effects	Behavioural and electroencephalographic effects	Neuroimaging
		• Sleep deprivation enhanced SWS, SWA and number of slow waves (< 2 Hz) more among 10R/10R carriers.	
Sleep loss is associated with increased availability of mGluR5 in human brain **Chapter 4**	The ∆mGluR5 availability was correlated to the ∆subjective sleepiness following sleep deprivation: • Globally • Anterior cingulate cortex • Insula • Medial temporal lobe • Parahippocampal gyrus • Striatum • Amygdala	n/a	The availability of mGluR5 was increased following sleep deprivation: • Globally • Anterior cingulate cortex • Insula • Medial temporal lobe • Parahippocampal gyrus • Striatum • Amygdala
Availability of mGluR5 is linked to EEG oscillations and to a distinct polymorphism of the fragile X gene **Chapter 5**	FMR1 odd vs even genotype: • Reduced habitual sleep duration among *FMR1* odd compared to even carriers. • Increased sleepiness symptoms throughout prolonged wakefulness among *FMR1* odd compared to even carriers • Reduced psychomotor performance (PVT percentile range) among *FMR1* odd compared to even carriers.	EEG oscillations correlated with the availability of mGluR5 • EEG slow-oscillations (< 1 Hz) in baseline and recovery. • EEG SWA (0.5 – 4.5 Hz) in baseline and recovery. • ∆EEG high SWA (2-4 Hz) is correlated to ∆mGluR5 availability following sleep deprivation. • ∆light sleep (stage 1) in PET scanner is correlated to ∆mGluR5 availability following sleep deprivation. • EEG slow oscillations and SWA correlated with regional mGluR5 availability (see Appendix 3).	FMR1 genotype modulated mGluR5 availability: • FMR1 odd had increased mGluR5 availability under baseline conditions when compared to even. • ∆mGluR5 with sleep deprivation was enhanced among FMR1 even carriers when compared to odd.

Topic	Subjective effects	Behavioural and electroencephalographic effects	Neuroimaging
		FMR1 odd vs even genotype • Healthy FMR1 odd carriers show a 1.9x increase in FMRP expression when compared to even. • FMR1 odd carriers have increase SWS% under baseline conditions when compared to even. • FMR1 even carriers show an increased ∆slow-oscillations compared to odd following sleep deprivation • FMR1 even carriers show an increased ∆SWA compared to odd following sleep deprivation • FMR1 even carriers show an increased ∆SWS% compared to odd following sleep deprivation	

References

Achermann P, Borbély AA (1997) Low-frequency (<1 Hz) oscillations in the human sleep electroencephalogram. Neuroscience 81:213–222.

Achermann P, Borbély AA (2011) Sleep homeostasis and models of sleep regulation. In: Principles and Practice of Sleep Medicine, 5 ed. (Kryger MH, Roth T, Dement WC, eds), pp 431–444. St. Louis: Saunders.

Achermann P, Dijk DJ, Brunner DP, Borbély AA (1993) A model of human sleep homeostasis based on EEG slow-wave activity: Quantitative comparison of data and simulations. Brain Res Bull 31:97–113.

Ahnaou A, Langlois X, Steckler T, Huysmans H, Riele Te P, Raeymakers L, Bartolome J, Drinkenburg W (2012) Pro-cognitive potential of metabotropic receptors (Glur5) blockade: Evidence from sleep behaviour and cortical network oscillations in rodents. J Sleep Res:21–80.

Akil M, Kolachana BS, Rothmond DA, Hyde TM, Weinberger DR, Kleinman JE (2003) Catechol-O-methyltransferase genotype and dopamine regulation in the human brain. J Neurosci 23:2008–2013.

Albrecht U, Eichele G (2003) The mammalian circadian clock. Curr Opin Genetics Dev 13:271–277.

Allen EG, He W, Yadav-Shah M, Sherman SL (2004) A study of the distributional characteristics of FMR1 transcript levels in 238 individuals. Hum Genet 114:439–447.

Ambrosius U, Lietzenmaier S, Wehrle R, Wichniak A, Kalus S, Winkelmann J, Bettecken T, Holsboer F, Yassouridis A, Friess E (2008) Heritability of sleep electroencephalogram. Biol Psychiat 64:344–348.

Ametamey SM, Kessler LJ, Honer M, Wyss MT, Buck A, Hintermann S, Auberson YP, Gasparini F, Schubiger PA (2006) Radiosynthesis and preclinical evaluation of 11C-ABP688 as a probe for imaging the metabotropic glutamate receptor subtype 5. J Nucl Med 47:698–705.

Ametamey SM, Treyer V, Streffer J, Wyss MT, Schmidt M, Blagoev M, Hintermann S, Auberson Y, Gasparini F, Fischer UC, Buck A (2007) Human PET studies of metabotropic glutamate receptor subtype 5 with 11C-ABP688. J Nucl Med 48:247–252.

Amos W, Driscoll E, Hoffman JI (2011) Candidate genes versus genome-wide associations: which are better for detecting genetic susceptibility to infectious disease? Proc R Soc B 278:1183–1188.

Amzica F, da Silva FHL (2012) Cellular substrates of brain rhythms. In: Niedermeyer's Electroencephalography, pp 33–63. Philadelphia: Lippincott Williams & Wilkins.

Andretic R, Kim Y-C, Jones FS, Han K-A, Greenspan RJ (2008) Drosophila D1 dopamine receptor mediates caffeine-induced arousal. PNAS 105:20392–20397.

Andreu N, Chalé J-J, Senard J-M, Thalamas C, Montastruc J-L, Rascol O (1999) L-dopa-induced sedation: A double-blind cross-over controlled study versus triazolam and placebo in healthy volunteers. Clin Neuropharmacol 22:15–23.

Anwyl R (2009) Metabotropic glutamate receptor-dependent long-term potentiation. Neuropharmacology 56:735–740.

Archer SN, Robilliard DL, Skene DJ, Smits M, Williams A, Arendt J, Schantz von M (2003) A length polymorphism in the circadian clock gene Per3 is linked to delayed sleep phase syndrome and extreme diurnal preference. SLEEP 26:413–415.

Arnulf I, Leu-Semenescu S (2009) Sleepiness in Parkinson's disease. Parkinsonism Rel Disord:101–104.

Aserinsky E, Kleitman N (1953) Regularly occurring periods of eye motility, and concomitant phenomena, during sleep. Science 118:273–274.

Ayala JE, Chen Y, Banko JL, Sheffler DJ, Williams R, Telk AN, Watson NL, Xiang Z, Zhang Y, Jones PJ, Lindsley CW, Olive MF, Conn PJ (2009) mGluR5 positive allosteric modulators facilitate both hippocampal LTP and LTD and enhance spatial learning. Neuropsychopharmacol 34:2057–2071.

Åkerstedt T, Gillberg M (1990) Subjective and objective sleepiness in the active individual. Int J Neurosci 52:29–37.

Bachmann V, Klaus F, Bodenmann S, Schäfer N, Brugger P, Huber S, Berger W, Landolt H-P (2012a) Functional ADA polymorphism increases sleep depth and reduces vigilant attention in humans. Cereb Cortex 22:962–970.

Bachmann V, Klein C, Bodenmann S, Schäfer N, Berger W, Brugger P, Landolt H-P (2012b) The BDNF Val66Met polymorphism modulates sleep intensity: EEG frequency- and state-specificity. SLEEP 35:335–344.

Baglioni C, Spiegelhalder K, Lombardo C, Riemann D (2010) Sleep and emotions: A focus on insomnia. Sleep Med Rev 14:227–238.

Bagni C, Greenough WT (2005) From mRNP trafficking to spine dysmorphogenesis: The roots of fragile X syndrome. Nat Rev Neurosci 6:376–387.

Barik S, de Beaurepaire R (2005) Dopamine D3 modulation of locomotor activity and sleep in the nucleus accumbens and in lobules 9 and 10 of the cerebellum in the rat. Prog Neuro-Psychoph 29:718–726.

Barker GC (2002) Microsatellite DNA: a tool for population genetic analysis. Trans R Soc Trop Med Hyg 96:S21–S24.

Barker GRI, Bashir ZI, Brown MW, Warburton EC (2006) A temporally distinct role for group I and group II metabotropic glutamate receptors in object recognition memory. Learn Mem 13:178–186.

Barkley RA, Smith KM, Fischer M, Navia B (2006) An examination of the behavioral and neuropsychological correlates of three ADHD candidate gene polymorphisms (DRD4 7+, DBH TaqI A2, and DAT1 40 bp VNTR) in hyperactive and normal children followed to adulthood. Am J Med Genet B 141B:487–498.

Basheer R, Strecker RE, Thakkar MM, McCarley RW (2004) Adenosine and sleep–wake regulation. Prog Neurobiol 73:379–396.

Bassell GJ, Warren ST (2008) Fragile X syndrome: Loss of local mRNA regulation alters synaptic development and function. Neuron 60:201–214.

Bear MF, Huber KM, Warren ST (2004) The mGluR theory of fragile X mental retardation. Trends Neurosci 27:370–377.

Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J (1961) An inventory for measuring depression. Arch Gen Psychiatry 4:561.

Ben A Oostra, Willemsen R (2009) FMR1: A gene with three faces. Biochim Biophys Acta 1790:467–477.

Benoit-Marand M, Jaber M, Gonon F (2000) Release and elimination of dopamine in vivo in mice lacking the dopamine transporter: Functional consequences. Eur J Neurosci 12:2985–2992.

Berger H (1929) Über das Elektrenkephalogramm des Menschen. Archiv f Psychiatrie 87:527–570.

Bersagliere A, Achermann P (2010) Slow oscillations in human non-rapid eye movement sleep electroencephalogram: Effects of increased sleep pressure. J Sleep Res 19:228–237.

Berson DM, Dunn FA, Takao M (2002) Phototransduction by retinal ganglion cells that set the circadian clock. Science 295:1070–1073.

Bes F, Wilde-Frenz J, Schulz H (1992) Application of the Tiredness Symptoms Scale during sleep deprivation. Sleep-Wake Research in the Netherlands:19–20.

Blackstone C (2009) Infantile parkinsonism-dystonia: a dopamine “transportopathy.” J Clin Invest 119:1455–1458.

Blake M (1967) Relationship between circadian rhythm of body temperature and introversion–extraversion. Nature:896–897.

Blasi G, Mattay VS, Bertolino A, Elvevåg B, Callicott JH, Das S, Kolachana BS, Egan MF, Goldberg TE, Weinberger DR (2005) Effect of catechol-O-methyltransferase val158met genotype on attentional control. J Neurosci 25:5038–5045.

Blethyn KL, Hughes SW, Tóth TI, Cope DW, Crunelli V (2006) Neuronal basis of the slow (<1Hz) oscillation in neurons of the nucleus reticularis thalami in vitro. J Neurosci 26:2474–2486.

Bloch KE, Schoch OD, Zhang JN, Russi EW (1999) German version of the Epworth sleepiness scale. Respiration 66:440–447.

Bodenmann S, Hohoff C, Freitag C, Deckert J, Rétey JV, Bachmann V, Landolt H-P (2012) Polymorphisms of ADORA2A modulate psychomotor vigilance and the effects of caffeine on neurobehavioural performance and sleep EEG after sleep deprivation. Brit J Pharmacol 165:1904–1913.

Bodenmann S, Landolt H-P (2010) Effects of modafinil on the sleep EEG depend on Val158Met genotype of COMT. SLEEP 33:1027–1035.

Bodenmann S, Rusterholz T, Dürr R, Stoll C, Bachmann V, Geissler E, Jaggi-Schwarz K, Landolt H-P (2009a) The functional Val158Met polymorphism of COMT predicts interindividual differences in brain alpha oscillations in young men. J Neurosci 29:10855–10862.

Bodenmann S, Xu S, Luhmann UFO, Arand M, Berger W, Jung HH, Landolt H-P (2009b) Pharmacogenetics of modafinil after sleep loss: Catechol-O-methyltransferase genotype modulates waking functions but not recovery sleep. Clin Pharmacol Ther 85:296–304.

Bodenmann SL (2009) Sleep-wake regulation in humans: Pharmacogenetic studies. Doctoral dissertation (Diss. ETH No. 18360).

Boonstra AM, Kooij JJS, Oosterlaan J, Sergeant JA, Buitelaar JK, van Someren EJW (2007) Hyperactive night and day? Actigraphy studies in adult ADHD: A baseline comparison and the effect of methylphenidate. SLEEP 30:433–442.

Borbély AA (1982) A two process model of sleep regulation. Hum Neurobiol 1:195–204.

Bourgeois JA, Coffey SM, Rivera SM, Hessl D, Gane LW, Tassone F, Greco C, Finucane B, Nelson L, Berry-Kravis E, Grigsby J, Hagerman PJ, Hagerman RJ (2009) A review of fragile X premutation disorders - expanding the psychiatric perspective. J Clin Psychiatry 70:852–862.

Boutrel B, Koob GF (2004) What keeps us awake: The neuropharmacology of stimulants and wakefulness-promoting medications. SLEEP 27:1181–1194.

Bremer F (1935) Cerveau isolé et physiologie du sommeil. C R Seances Soc Biol Fil 118:1235–1241.

Buckelmüller J, Landolt H-P, Stassen HH, Achermann P (2006) Trait-like individual differences in the human sleep electroencephalogram. Neuroscience 138:351–356.

Bunney BG, Bunney WE (2012) Rapid-acting antidepressant strategies: mechanisms of action. Int J Neuropsychoph 15:695–713.

Burger C, Deschwanden A, Ametamey S, Johayem A, Mancosu B, Wyss M, Hasler G, Buck A (2010) Evaluation of a bolus/infusion protocol for 11C-ABP688, a PET tracer for mGluR5. Nucl Med Biol 37:845–851.

Bushey D, Tononi G, Cirelli C (2009) The drosophila fragile X mental retardation gene regulates sleep need. J Neurosci 29:1948–1961.

Bushey D, Tononi G, Cirelli C (2011) Sleep and synaptic homeostasis: Structural evidence in

drosophila. Science 332:1576–1581.

Buzsáki G, Draguhn A (2004) Neuronal oscillations in cortical networks. Science 304:1926–1929.

Campbell SS, Tobler I (1984) Animal sleep: a review of sleep duration across phylogeny. Neurosci Biobehav Rev 8:269–300.

Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, Richardson JA, Williams SC, Xiong Y, Kisanuki Y, Fitch TE, Nakazato M, Hammer RE, Saper CB, Yanagisawa M (1999) Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell 98:437–451.

Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, Kolachana BS, Hyde TM, Herman MM, Apud J, Egan MF, Kleinman JE, Weinberger DR (2004) Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): Effects on mRNA, protein, and enzyme activity in postmortem human brain. Am J Hum Genet 75:807–821.

Cheon K-A, Ryu YH, Kim Y-K, Namkoong K, Kim C-H, Lee J (2003) Dopamine transporter density in the basal ganglia assessed with [123I]IPT SPET in children with attention deficit hyperactivity disorder. Eur J Nucl Med Mol Imaging 30:306–311.

Chuah YML, Venkatraman V, Dinges DF, Chee MWL (2006) The neural basis of interindividual variability in inhibitory efficiency after sleep deprivation. J Neurosci 26:7156–7162.

Ciliax BJ, Drash GW, Staley JK, Haber S, Mobley CJ, Miller GW, Mufson EJ, Mash DC, Levey AI (1999) Immunocytochemical localization of the dopamine transporter in human brain. J Comp Neurol 409:38–56.

Ciliax BJ, Heilman C, Demchyshyn LL, Pristupa ZB, Ince E, Hersch SM, Niznik HB, Levey AI (1995) The dopamine transporter: Immunochemical characterization and localization in brain. J Neurosci 15:1714–1723.

Cirelli C, Gutierrez CM, Tononi G (2004) Extensive and divergent effects of sleep and wakefulness on brain gene expression. Neuron 41:35–43.

Cirelli C, Tononi G (2000) Differential expression of plasticity-related genes in waking and sleep and their regulation by the noradrenergic system. J Neurosci 20:9187–9194.

Cirelli C, Tononi G (2008) Is sleep essential? PLoS Biol 6:e216.

Ciruela F, Casadó V, Rodrigues RJ, Lujan R, Burgueño J, Canals M, Borycz J, Rebola N, Goldberg SR, Mallol J, Cortés A, Canela EI, López-Giménez JF, Milligan G, Lluis C, Cunha RA, Ferré S, Franco R (2006) Presynaptic control of striatal glutamatergic neurotransmission by adenosine A1-A2A receptor heteromers. J Neurosci 26:2080–2087.

Cobb SR, Bulters DO, Davies CH (2000) Coincident activation of mGluRs and mAChRs imposes theta frequency patterning on synchronised network activity in the hippocampal CA3 region. Neuropharmacology 39:1933–1942.

Cohen-Zion M, Ancoli-Israel S (2004) Sleep in children with attention-deficit hyperactivity disorder (ADHD): A review of naturalistic and stimulant intervention studies. Sleep Med Rev 8:379–402.

Collingridge GL, Isaac JTR, Wang YT (2004) Receptor trafficking and synaptic plasticity. Nat Rev Neurosci 5:952–962.

Comella CL (2008) Sleep disorders in Parkinson's disease. Curr Treat Options Neurol 10:215–221.

Contreras D, Steriade M (1995) Cellular basis of EEG slow rhythms: a study of dynamic corticothalamic relationships. J Neurosci 15:604–622.

Cooley JW, Tukey JW (1965) An Algorithm for the Machine Calculation of Complex Fourier Series. Math Comput 19:297–301.

Cortese S, Faraone SV, Konofal E, Lecendreux M (2009) Sleep in children with attention-deficit/hyperactivity disorder: Meta-analysis of subjective and objective studies. J Am Acad Child

Adolesc Psychiatry 48:894–908.

Costa A, Riedel M, Müller U, Möller H-J, Ettinger U (2011) Relationship between SLC6A3 genotype and striatal dopamine transporter availability: A meta-analysis of human single photon emission computed tomography studies. Synapse 65:998–1005.

Crunelli V, Hughes SW (2010) The slow (<1 Hz) rhythm of non-REM sleep: A dialogue between three cardinal oscillators. Nature Neurosci 13:9–17.

Cummings CJ, Zoghbi HY (2000) Trinucleotide repeats: Mechanisms and pathophysiology. Annu Rev Genom Human Genet 1:281–328.

D'Ascenzo M, Fellin T, Terunuma M, Revilla-Sanchez R, Meaney DF, Auberson YP, Moss SJ, Haydon PG (2007) mGluR5 stimulates gliotransmission in the nucleus accumbens. PNAS 104:1995–2000.

Daan S, Beersma DG, Borbély AA (1984) Timing of human sleep: Recovery process gated by a circadian pacemaker. Am J Physiol 246:R161–R183.

Dahan L, Astier B, Vautrelle N, Urbain N, Kocsis B, Chouvet G (2007) Prominent burst firing of dopaminergic neurons in the ventral tegmental area during paradoxical sleep. Neuropsychopharmacol 32:1232–1241.

Dang-Vu TT, Desseilles M, Laureys S, Degueldre C, Perrin F, Phillips C, Maquet P, Peigneux P (2005) Cerebral correlates of delta waves during non-REM sleep revisited. Neuroimage 28:14–21.

Dang-Vu TT, Schabus M, Desseilles M, Albouy G, Boly M, Darsaud A, Gais S, Rauchs G, Sterpenich V, Vandewalle G, Carrier J, Moonen G, Balteau E, Degueldre C, Luxen A, Phillips C, Maquet P (2008) Spontaneous neural activity during human slow wave sleep. PNAS 105:15160–15165.

Dang-Vu TT, Schabus M, Desseilles M, Sterpenich V, Bonjean M, Maquet P (2010) Functional neuroimaging insights into the physiology of human sleep. SLEEP 33:1589.

Darlow JM, Leach DR (1995) The effects of trinucleotide repeats found in human inherited disorders on palindrome inviability in Escherichia coli suggest hairpin folding preferences in vivo. Genetics 141:825–832.

Darlow JM, Leach DR (1998a) Evidence for two preferred hairpin folding patterns in d(CGG).d(CCG) repeat tracts in vivo. J Mol Biol 275:17–23.

Darlow JM, Leach DR (1998b) Secondary structures in d(CGG) and d(CCG) repeat tracts. J Mol Biol 275:3–16.

Dash MB, Douglas CL, Vyazovskiy VV, Cirelli C, Tononi G (2009) Long-term homeostasis of extracellular glutamate in the rat cerebral cortex across sleep and waking states. J Neurosci 29:620–629.

Dauvilliers Y, Neidhart E, Lecendreux M, Billiard M, Tafti M (2001) MAO-A and COMT polymorphisms and gene effects in narcolepsy. Mol Psychiatry 6:367–372.

de Gennaro L, Marzano C, Fratello F, Moroni F, Pellicciari MC, Ferlazzo F, Costa S, Couyoumdjian A, Curcio G, Sforza E, Malafosse A, Finelli LA, Pasqualetti P, Ferrara M, Bertini M, Rossini PM (2008) The electroencephalographic fingerprint of sleep is genetically determined: A twin study. Annals of Neurology 64:455–460.

Deese J (1959) On the prediction of occurrence of particular verbal intrusions in immediate recall. J Exp Psychol 58:17–22.

DeLorenzo C, Kumar JD, Mann JJ, Parsey RV (2011) In vivo variation in metabotropic glutamate receptor subtype 5 binding using positron emission tomography and [11C]ABP688. J Cereb Blood Flow Metab 31:2169–2180.

Dement W, Kleitman N (1957) Cyclic variations in EEG during sleep and their relation to eye movements, body motility, and dreaming. Electroencephalogr Clin Neurophysiol 9:673–690.

Deschwanden A, Karolewicz B, Feyissa AM, Treyer V, Ametamey SM, Johayem A, Burger C, Auberson YP, Sovago J, Stockmeier CA (2011) Reduced Metabotropic Glutamate Receptor 5 Density in Major Depression Determined by [11C] ABP688 Positron Emission Tomography and Postmortem Study. Am J Psychiat 168:727.

Diekelmann S, Born J (2010) The memory function of sleep. Nat Rev Neurosci 11:114–126.

Diekelmann S, Büchel C, Born J, Rasch B (2011) Labile or stable: opposing consequences for memory when reactivated during waking and sleep. Nature Neurosci 14:381–386.

Dingemanse J, Jorga K, Zurcher G, Schmitt M, Sedek G, Prada M, Brummelen PV (1995) Pharmacokinetic□pharmacodynamic interaction between the COMT inhibitor tolcapone and single□dose levodopa. Brit J Clin Pharmaco 40:253–262.

Dinges DF, Powell JW (1985) Microcomputer analyses of performance on a portable, simple visual RT task during sustained operations. Behav Res Meth Instr 17:652–655.

Dougherty DD, Bonab AA, Spencer TJ, Rauch SL, Madras BK, Fischman AJ (1999) Dopamine transporter density in patients with attention deficit hyperactivity disorder. Lancet 354:2132–2133.

Dölen G, Bear MF (2008) Role for metabotropic glutamate receptor 5 (mGluR5) in the pathogenesis of fragile X syndrome. J Physiol 586:1503–1508.

Dölen G, Osterweil E, Rao BSS, Smith GB, Auerbach BD, Chattarji S, Bear MF (2007) Correction of Fragile X Syndrome in mice. Neuron 56:955–962.

Dreher J-C, Schmidt PJ, Kohn P, Furman D, Rubinow D, Berman KF (2007) Menstrual cycle phase modulates reward-related neural function in women. PNAS 104:2465–2470.

Dresel S, Krause J, Krause KH, LaFougere C (2000) Attention deficit hyperactivity disorder: Binding of [99mTc] TRODAT-1 to the dopamine transporter before and after methylphenidate treatment. Eur J Nucl Med.

Dressendörfer RA, Kirschbaum C, Rohde W, Stahl F, Strasburger CJ (1992) Synthesis of a cortisol-biotin conjugate and evaluation as a tracer in an immunoassay for salivary cortisol measurement. J Steroid Biochem Mol Biol 43:683–692.

Durmer JS, Dinges DF (2005) Neurocognitive consequences of sleep deprivation. Semin Neurol 25:117–129.

Economo Von C (1930) Sleep as a problem of localization. J Nerv Ment Dis 71:249–259.

Edlow BL, Takahashi E, Wu O, Benner T, Dai G, Bu L, Grant PE, Greer DM, Greenberg SM, Kinney HC, Folkerth RD (2012) Neuroanatomic connectivity of the human ascending arousal system critical to consciousness and its disorders. J Neuropathol Exp Neurol 71:531–546.

Elmenhorst D, Meyer PT, Winz OH, Matusch A, Ermert J, Coenen HH, Basheer R, Haas HL, Zilles K, Bauer A (2007) Sleep deprivation increases A1 adenosine receptor binding in the human brain: A positron emission tomography study. J Neurosci 27:2410–2415.

Elmenhorst D, Minuzzi L, Aliaga A, Rowley J, Massarweh G, Diksic M, Bauer A, Rosa-Neto P (2010) In vivo and in vitro validation of reference tissue models for the mGluR(5) ligand [(11)C]ABP688. J Cereb Blood Flow Metab 30:1538–1549.

Faraguna U, Vyazovskiy VV, Nelson AB, Tononi G, Cirelli C (2008) A causal role for brain-derived neurotrophic factor in the homeostatic regulation of sleep. J Neurosci 28:4088–4095.

Feinberg I, Campbell IG (1993) Ketamine administration during waking increases delta EEG intensity in rat sleep. Neuropsychopharmacol 9:41–48.

Fellin T, Pascual O, Gobbo S, Pozzan T, Haydon PG, Carmignoto G (2004) Neuronal synchrony mediated by astrocytic glutamate through activation of extrasynaptic NMDA receptors. Neuron 43:729–743.

Ferré S, Ciruela F, Woods AS, Lluis C, Franco R (2007) Functional relevance of neurotransmitter receptor heteromers in the central nervous system. Trends Neurosci 30:440–446.

Ferré S, Karcz-Kubicha M, Hope BT, Popoli P, Burgueño J, Gutiérrez MA, Casadó V, Fuxe K, Goldberg SR, Lluis C (2002) Synergistic interaction between adenosine A2A and glutamate mGlu5 receptors: Implications for striatal neuronal function. PNAS 99:11940–11945.

Fisone G, Borgkvist A, Usiello A (2004) Caffeine as a psychomotor stimulant: Mechanism of action. Cell Mol Life Sci 61:857–872.

Fontinha BM, Diógenes MJ, Ribeiro JA, Sebastião AM (2008) Enhancement of long-term potentiation by brain-derived neurotrophic factor requires adenosine A2A receptor activation by endogenous adenosine. Neuropharmacology 54:924–933.

Fort P, Bassetti CL, Luppi PH (2009) Alternating vigilance states: New insights regarding neuronal networks and mechanisms. Eur J Neurosci 29:1741–1753.

Francis LJ, Lewis CA, Ziebertz H-G (2006) The short-form revised Eysenck personality questionnaire (EPQR-S): A German edition. Soc Behav Personal 34:197–204.

Frank MG (2012) Erasing synapses in sleep: Is it time to be SHY? Neural Plast 2012:1–15.

Franke B et al. (2010) Multicenter analysis of the SLC6A3/DAT1 VNTR haplotype in persistent ADHD suggests differential involvement of the gene in childhood and persistent ADHD. Neuropsychopharmacol 35:656–664.

Franken P, Chollet D, Tafti M (2001) The homeostatic regulation of sleep need is under genetic control. J Neurosci 21:2610–2621.

Fredholm BB, Bättig K, Holmén J, Nehlig A, Zvartau EE (1999) Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol Rev 51:83–133.

Fuke S, Suo S, Takahashi N, Koike H, Sasagawa N, Ishiura S (2001) The VNTR polymorphism of the human dopamine transporter (DAT1) gene affects gene expression. Pharmacogenomics J 1:152–156.

Fuller P, Sherman D, Pedersen NP, Saper CB, Lu J (2011) Reassessment of the structural basis of the ascending arousal system. J Comp Neurol 519:933–956.

Fuxe K, Ferré S, Canals M, Torvinen M, Terasmaa A, Marcellino D, Goldberg SR, Staines W, Jacobsen KX, Lluis C (2005) Adenosine A2A and dopamine D2 heteromeric receptor complexes and their function. J Mol Neurosci 26:209–220.

Gallopin T, Luppi PH, Cauli B, Urade Y, Rossier J, Hayaishi O, Lambolez B, Fort P (2005) The endogenous somnogen adenosine excites a subset of sleep-promoting neurons via A2A receptors in the ventrolateral preoptic nucleus. Neuroscience 134:1377–1390.

Galvão ACS, Krüger RC, Campagnolo PDB, Mattevi VS, Vitolo MR, Almeida S (2012) Association of MAOA and COMT gene polymorphisms with palatable food intake in children. J Nutr Biochem 23:272–277.

Gasparini F, Bilbe G, Gomez-Mancilla B, Spooren W (2008) mGluR5 antagonists: Discovery, characterization and drug development. Curr Opin Drug Discov Devel 11:655–665.

Geyer H (1937) Ueber den Schlaf von Zwillingen. Mol Gen Genet 73:524–527.

Giros B, Caron MG (1993) Molecular characterization of the dopamine transporter. Trends Pharmacol Sci 14:43–49.

Giros B, Jaber M, Jones SR, Wightman RM, Caron MG (1996) Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature 379:606–612.

Gooley JJ, Lu J, Chou TC, Scammell TE, Saper CB (2001) Melanopsin in cells of origin of the retinohypothalamic tract. Nature Neurosci 4:1165.

Graw P, Kräuchi K, Knoblauch V, Wirz-Justice A, Cajochen C (2004) Circadian and wake-dependent modulation of fastest and slowest reaction times during the psychomotor vigilance task. Physiol Behav 80:695–701.

Greenspan RJ, Tononi G, Cirelli C, Shaw PJ (2001) Sleep and the fruit fly. Trends Neurosci 24:142–145.

Guindalini C, Martins RC, Andersen ML, Tufik S (2010) Influence of genotype on dopamine transporter availability in human striatum and sleep architecture. Psychiat Res 179:238–240.

Hays SA, Huber KM, Gibson JR (2011) Altered neocortical rhythmic activity states in Fmr1 KO mice are due to enhanced mGluR5 signaling and involve changes in excitatory circuitry. J Neurosci 31:14223–14234.

Hefti K, Holst SC, Sovago J, Bachmann V, Buck A, Ametamey SM, Scheidegger M, Berthold T, Gomez-Mancilla B, Seifritz E, Landolt H-P (2013) Increased metabotropic glutamate receptor subtype 5 availability in human brain after one night without sleep. Biol Psychiat 73:161–168.

Heinz A, Goldman D, Jones DW, Palmour R, Hommer D, Gorey JG, Lee KS, Linnoila M, Weinberger DR (2000) Genotype influences in vivo dopamine transporter availability in human striatum. Neuropsychopharmacol 22:133–139.

Hobson JA (2005) Sleep is of the brain, by the brain and for the brain. Nature 437:1254–1256.

Hobson JA, Pace-Schott EF (2002) The cognitive neuroscience of sleep: neuronal systems, consciousness and learning. Nat Rev Neurosci 3:679–693.

Holst SC, Bersagliere A, Bachmann V, Achermann P, Landolt H-P (2012) A genetic polymorphism of dopamine transporter modulates EEG markers of sleep homeostasis and effects of caffeine after sleep deprivation. J Sleep Res:21–80.

Horne JA, Ostberg O (1976) A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. Int J Chronobiology 4:97–110.

Hosák L (2007) Role of the COMT gene Val158Met polymorphism in mental disorders: A review. Eur Psychiatry 22:276–281.

Huang Z-L, Qu W-M, Eguchi N, Chen J-F, Schwarzschild MA, Fredholm BB, Urade Y, Hayaishi O (2005) Adenosine A2A, but not A1, receptors mediate the arousal effect of caffeine. Nature Neurosci 8:858–859.

Huber KM, Gallagher SM, Warren ST, Bear MF (2002) Altered synaptic plasticity in a mouse model of fragile X mental retardation. PNAS 99:7746–7750.

Huber R, Ghilardi MF, Massimini M, Tononi G (2004) Local sleep and learning. Nature 430:78–81.

Huber R, Mäki H, Rosanova M, Casarotto S, Canali P, Casali AG, Tononi G, Massimini M (2012) Human cortical excitability increases with time awake. Cereb Cortex.

Huber R, Tononi G, Cirelli C (2007) Exploratory behavior, cortical BDNF expression, and sleep homeostasis. SLEEP 30:129–139.

Hughes SW, Cope DW, Blethyn KL, Crunelli V (2002) Cellular mechanisms of the slow (<1 Hz) oscillation in thalamocortical neurons in vitro. Neuron 33:947–958.

Hulka LM, Treyer V, Scheidegger M, Preller KH, Vonmoos M, Baumgartner MR, Johayem A, Ametamey SM, Buck A, Seifritz E, Quednow BB (2013) Smoking but not cocaine use is associated with lower cerebral metabotropic glutamate receptor 5 density in humans. Mol. Psychiatry :1–8.

Huysmans H, Ahnaou A, Shaban H, Langlois X, P TR, Raeymaekers L, Steckler T, Bartolome J, Drinkenburg W (2012) Antagonism versus positive allosteric modulation of metabotropic glutamate (mGluR5) receptors: EEG and sleep-wake behavior indices for potential pro-cognitive drug properties in rodents. Unpublished observations.

Irwin SA, Patel B, Idupulapati M, Harris JB, Crisostomo RA, Larsen BP, Kooy F, Willems PJ, Cras P, Kozlowski PB (2001) Abnormal dendritic spine characteristics in the temporal and visual cortices of patients with fragile X syndrome: a quantitative examination. Am J Hum Genet 98:161–167.

Isaac SO, Berridge CW (2003) Wake-promoting actions of dopamine D1 and D2 receptor stimulation. J Pharmacol Exp Ther 307:386–394.

Izumi Y, Zorumski CF (2012) NMDA receptors, mGluR5, and endocannabinoids are involved in a cascade leading to hippocampal long-term depression. Neuropsychopharmacol 37:609–617.

Jaber M, Jones S, Giros B, Caron MG (2004) The dopamine transporter: A crucial component regulating dopamine transmission. Mov Disorders 12:629–633.

Jacobsen LK, Staley JK, Zoghbi SS, Seibyl JP, Kosten TR, Innis RB, Gelernter J (2000) Prediction of dopamine transporter binding availability by genotype: A preliminary report. Am J Psychiat 157:1700–1703.

Johns MW (1991) A new method for measuring daytime sleepiness: the Epworth sleepiness scale. SLEEP 14:540–545.

Johns MW (2000) Sensitivity and specificity of the multiple sleep latency test (MSLT), the maintenance of wakefulness test and the epworth sleepiness scale: failure of the MSLT as a gold standard. J Sleep Res 9:5–11.

Jones SR, Gainetdinov RR, Jaber M, Giros B, Wightman RM, Caron MG (1998) Profound neuronal plasticity in response to inactivation of the dopamine transporter. PNAS 95:4029–4034.

Jung SS, Sung KW, Lee SE, Shin HK (2011) Capsaicin prevents the hyperalgesia induced by peripheral group I mGluRs activation. Neurosci Lett 500:197–201.

Kaasinen V, Aalto S, Någren K, Rinne JO (2004) Dopaminergic effects of caffeine in the human striatum and thalamus. Neuroreport 15:281–285.

Kahlig KM, Javitch JA, Galli A (2004) Amphetamine regulation of dopamine transport. J Biol Chem 279:8966–8975.

Kammermeier PJ, Worley PF (2007) Homer 1a uncouples metabotropic glutamate receptor 5 from postsynaptic effectors. PNAS 104:6055–6060.

Kandel E, Schwartz J, Jessell T (2000) Principles of Neural Science, 4 ed. New York: McGraw-Hill Professional.

Kang AM, Palmatier MA, Kidd KK (1999) Global variation of a 40-bp VNTR in the 3'-untranslated region of the dopamine transporter gene (SLC6A3). Biol Psychiat 46:151–160.

Kauer JA, Malenka RC (2007) Synaptic plasticity and addiction. Nat Rev Neurosci 8:844–858.

Kemp A, Manahan-Vaughan D (2007) Hippocampal long-term depression: Master or minion in declarative memory processes? Trends Neurosci 30:111–118.

Kereszturi E, Tarnok Z, Bognar E, Lakatos K, Farkas L, Gadoros J, Sasvari-Szekely M, Nemoda Z (2008) Catechol-O-methyltransferase Val158Met polymorphism is associated with methylphenidate response in ADHD children. Am J Med Genet B 147B:1431–1435.

Kilty JE, Lorang D, Amara SG (1991) Cloning and expression of a cocaine-sensitive rat dopamine transporter. Science 254:578–579.

Krause J, Dresel SH, Krause K-H, La Fougère C, Zill P, Ackenheil M (2006) Striatal dopamine transporter availability and DAT-1 gene in adults with ADHD: No higher DAT availability in patients with homozygosity for the 10-repeat allele. World J Biol Psychiatry 7:152–157.

Kripke DF, Garfinkel L, Wingard DL, Klauber MR, Marler MR (2002) Mortality associated with sleep duration and insomnia. Arch Gen Psychiatry 59:131–136.

Kume K, Kume S, Park SK, Hirsh J, Jackson FR (2005) Dopamine is a regulator of arousal in the fruit fly. J Neurosci 25:7377–7384.

Kurian MA, Zhen J, Cheng S-Y, Li Y, Mordekar SR, Jardine P, Morgan NV, Meyer E, Tee L, Pasha S, Wassmer E, Heales SJR, Gissen P, Reith MEA, Maher ER (2009) Homozygous loss-of-function mutations in the gene encoding the dopamine transporter are associated with infantile parkinsonism-dystonia. J Clin Invest 119:1595–1603.

Landolt H-P (2008) Sleep homeostasis: A role for adenosine in humans? Biochem Pharmacol 75:2070–2079.

Landolt H-P, Rétey JV, Tönz K, Gottselig JM, Khatami R, Buckelmüller I, Achermann P (2004) Caffeine attenuates waking and sleep electroencephalographic markers of sleep homeostasis in humans. Neuropsychopharmacol 29:1933–1939.

Landolt H-P, Werth E, Borbély AA, Dijk DJ (1995) Caffeine intake (200 mg) in the morning affects human sleep and EEG power spectra at night. Brain Res 675:67-74.

Lazarus M, Chen J-F, Urade Y, Huang Z-L (2013) Role of the basal ganglia in the control of sleep and wakefulness. Curr Opin Neurobiol:1–6.

Lazarus M, Huang Z-L, Lu J, Urade Y, Chen J-F (2012) How do the basal ganglia regulate sleep–wake behavior? Trends Neurosci 35:723–732.

Lázár AS, Slak A, Lo JC-Y, Santhi N, Schantz von M, Archer SN, Groeger JA, Dijk D-J (2012) Sleep, diurnal preference, health, and psychological well-being: A prospective single-allelic-variation study. Chronobiol Int 29:131–146.

Lee MG, Hassani OK, Jones BE (2005) Discharge of identified orexin/hypocretin neurons across the sleep-waking cycle. J Neurosci 25:6716–6720.

Lees AJ, Blackburn NA, Campbell VL (1988) The nighttime problems of Parkinson's disease. Clin Neuropharmacol 11:512–519.

Legutko B, Szewczyk B, Pomierny-Chamioło L, Nowak G, Pilc A (2006) Effect of MPEP treatment on brain-derived neurotrophic factor gene expression. Pharmacol Rep 58:427–430.

Lewis DA, Melchitzky DS, Sesack SR, Whitehead RE, Auh S, Sampson A (2001) Dopamine transporter immunoreactivity in monkey cerebral cortex: Regional, laminar, and ultrastructural localization. J Comp Neurol 432:119–136.

Léna I, Parrot S, Deschaux O, Muffat-Joly S, Sauvinet V, Renaud B, Suaud-Chagny MF, Gottesmann C (2005) Variations in extracellular levels of dopamine, noradrenaline, glutamate, and aspartate across the sleep--wake cycle in the medial prefrontal cortex and nucleus accumbens of freely moving rats. J Neurosci Res 81:891–899.

Li CS, Chung S, Lu DP, Cho YK (2012) Descending projections from the nucleus accumbens shell suppress activity of taste-responsive neurons in the hamster parabrachial nuclei. J Neurophysiol 108:1288–1298.

Lin J-J, Yueh K-C, Chang D-C, Chang C-Y, Yeh Y-H, Lin S-Z (2003) The homozygote 10-copy genotype of variable number tandem repeat dopamine transporter gene may confer protection against Parkinson's disease for male, but not to female patients. J Neurol Sci 209:87–92.

Lovinger DM (2010) Neurotransmitter roles in synaptic modulation, plasticity and learning in the dorsal striatum. Neuropharmacology 58:951–961.

Lu J, Jhou TC, Saper CB (2006) Identification of wake-active dopaminergic neurons in the ventral periaqueductal gray matter. J Neurosci 26:193–202.

Lu Y-M, Jia Z, Janus C, Henderson JT, Gerlai R, Wojtowicz JM, Roder JC (1997) Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. J Neurosci 17:5196–5205.

Lujan R, Nusser Z, Roberts JD, Shigemoto R, Somogyi P (1996) Perisynaptic location of metabotropic glutamate receptors mGluR1 and mGluR5 on dendrites and dendritic spines in the rat hippocampus. Eur J Neurosci 8:1488–1500.

Maddalena A, Richards CS, McGinniss MJ, Brothman A, Desnick RJ, Grier RE, Hirsch B, Jacky P, McDowell GA, Popovich B, Watson M, Wolff DJ (2001) Technical standards and guidelines for fragile X: the first of a series of disease-specific supplements to the Standards and Guidelines for Clinical Genetics Laboratories of the American College of Medical Genetics. Quality Assurance Subcommittee of the Laboratory Practice Committee. Genet Med 3:200–205.

Madras BK, Xie Z, Lin Z, Jassen A, Panas H, Lynch L, Johnson R, Livni E, Spencer TJ, Bonab AA, Miller GM, Fischman AJ (2006) Modafinil occupies dopamine and norepinephrine transporters in vivo and modulates the transporters and trace amine activity in vitro. J Pharmacol Exp Ther 319:561–569.

Maher B (2008) Poll results: Look who's doping. Nature 452:674–675.

Malenka RC, Bear MF (2004) LTP and LTD: An Embarrassment of Riches. Neuron 44:5–21.

Malhotra AK, Kestler LJ, Mazzanti C, Bates JA, Goldberg TE, Goldman D (2002) A functional polymorphism in the COMT gene and performance on a test of prefrontal cognition. Am J Psychiat 159:652–654.

Malys N, McCarthy JEG (2011) Translation initiation: Variations in the mechanism can be anticipated. Cell Mol Life Sci 68:991–1003.

Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, McCarthy MI, Ramos EM, Cardon LR, Chakravarti A (2009) Finding the missing heritability of complex diseases. Nature 461:747–753.

Maquet P, Degueldre C, Delfiore G, Aerts J, Péters J-M, Luxen A, Franck G (1997) Functional neuroanatomy of human slow wave sleep. J Neurosci 17:2807–2812.

Maret S, Dorsaz S, Gurcel L, Pradervand S, Petit B, Pfister C, Hagenbuchle O, O'Hara BF, Franken P, Tafti M (2007) Homer1a is a core brain molecular correlate of sleep loss. PNAS 104:20090–20095.

Marshall L, Helgadóttir H, Mölle M, Born J (2006) Boosting slow oscillations during sleep potentiates memory. Nature 444:610–613.

Marshall L, Kirov R, Brade J, Mölle M, Born J (2011) Transcranial Electrical Currents to Probe EEG Brain Rhythms and Memory Consolidation during Sleep in Humans Dickson C, ed. PLoS ONE 6:e16905.

Mascetti L, Foret A, Bourdiec AS-L, Muto V, Kussé C, Jaspar M, Matarazzo L, Dang-Vu T, Schabus M, Maquet P (2011) Spontaneous neural activity during human non-rapid eye movement sleep. Prog Brain Res 193:111–118.

Massimini M, Ferrarelli F, Esser SK, Riedner BA, Huber R, Murphy M, Peterson MJ, Tononi G (2007) Triggering sleep slow waves by transcranial magnetic stimulation. PNAS 104:8496–8501.

Massimini M, Huber R, Ferrarelli F, Hill S (2004) The sleep slow oscillation as a traveling wave. J Neurosci 24:6862– 6870.

McCormick DA, Pape HC (1990) Properties of a hyperpolarization-activated cation current and its role in rhythmic oscillation in thalamic relay neurones. J Physiol 431:291–318.

McNair DM, Lorr M, Doppleman LF (1971) *Edits Manual for the Profile of Mood States (POMS)*. Educational and Industrial Testing Service, San Diego, CA.

Meyer-Lindenberg A, Kohn PD, Kolachana B, Kippenhan S, McInerney-Leo A, Nussbaum R, Weinberger DR, Berman KF (2005) Midbrain dopamine and prefrontal function in humans: Interaction and modulation by COMT genotype. Nature Neurosci 8:594–596.

Micallef J, Rey M, Eusebio A, Audebert C, Rouby F, Jouve E, Tardieu S, Blin O (2009) Antiparkinsonian

drug-induced sleepiness: A double-blind placebo-controlled study of L-dopa, bromocriptine and pramipexole in healthy subjects. Brit J Clin Pharmaco 67:333–340.

Michalon A, Sidorov M, Ballard TM, Ozmen L, Spooren W, Wettstein JG, Jaeschke G, Bear MF, Lindemann L (2012) Chronic pharmacological mGlu5 inhibition corrects Fragile X in adult mice. Neuron 74:49–56.

Michelhaugh SK, Fiskerstrand C, Lovejoy E, Bannon MJ, Quinn JP (2001) The dopamine transporter gene (SLC6A3) variable number of tandem repeats domain enhances transcription in dopamine neurons. J Neurochem 79:1033–1038.

Mignot E, Nishino S, Guilleminault C, Dement WC (1994) Modafinil binds to the dopamine uptake carrier site with low affinity. SLEEP 17:436–437.

Miller JD, Farber J, Gatz P, Roffwarg H, German DC (1983) Activity of mesencephalic dopamine and non-dopamine neurons across stages of sleep and waking in the rat. Brain Res 273:133–141.

Minzenberg MJ (2012) Pharmacotherapy for attention-deficit/hyperactivity disorder: From cells to circuits. Neurotherapeutics 9:610–621.

Minzenberg MJ, Carter CS (2008) Modafinil: A review of neurochemical actions and effects on cognition. Neuropsychopharmacol 33:1477–1502.

Mirkin SM (2007) Expandable DNA repeats and human disease. Nature 447:932–940.

Mitchell HA, Bogenpohl JW, Liles LC, Epstein MP, Bozyczko-Coyne D, Williams M, Weinshenker D (2008) Behavioral responses of dopamine beta-hydroxylase knockout mice to modafinil suggest a dual noradrenergic-dopaminergic mechanism of action. Pharmacol Biochem Behav 91:217–222.

Monti JM, Monti D (2007) The involvement of dopamine in the modulation of sleep and waking. Sleep Med Rev 11:113–133.

Moore RY, Eichler VB (1972) Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Res 42:201–206.

Morón JA, Brockington A, Wise RA, Rocha BA, Hope BT (2002) Dopamine uptake through the norepinephrine transporter in brain regions with low levels of the dopamine transporter: evidence from knock-out mouse lines. J Neurosci 22:389–395.

Moruzzi G, Magoun HW (1949) Brain stem reticular formation and activation of the EEG. Electroencephalogr Clin Neurophysiol 1:455–473.

Mukovski M, Chauvette S, Timofeev I, Volgushev M (2007) Detection of active and silent states in neocortical neurons from the field potential signal during slow-wave sleep. Cereb Cortex 17:400–414.

Murphy M, Riedner BA, Huber R, Massimini M, Ferrarelli F, Tononi G (2009) Source modeling sleep slow waves. PNAS 106:1608–1613.

Muzur A, Pace-Schott EF, Hobson JA (2002) The prefrontal cortex in sleep. Trends Cogn Sci 6:475–481.

Nagahara AH, Tuszynski MH (2011) Potential therapeutic uses of BDNF in neurological and psychiatric disorders. Nat Rev Drug Discov 10:209–219.

Nakamura Y, Koyama K, Matsushima M (1998) VNTR (variable number of tandem repeat) sequences as transcriptional, translational, or functional regulators. J Hum Genet 43:149–152.

Nakanishi H, Sun Y, Nakamura RK, Mori K, Ito M, Suda S, Namba H, Storch FI, Dang TP, Mendelson W, Mishkin M, Kennedy C, Gillin JC, Smith CB, Sokoloff L (1997) Positive correlations between cerebral protein synthesis rates and deep sleep in Macaca mulatta. Eur J Neurosci 9:271–279.

Need AC, Ahmadi KR, Spector TD, Goldstein DB (2006) Obesity is associated with genetic variants that alter dopamine availability. Ann Hum Genet 70:293–303.

Nelson DL, Cox MM (2008) G protein-coupled receptors and second messengers. In: Lehninger Principles of Biochemistry, 5 ed. W. H. New York: Freeman and company.

Nestler EJ, Hyman SE, Malenka RC (2009a) Sleep and arousal. In: Molecular Neuropharmacology, 2nd ed. pp 289–312. New York: McGraw-Hill.

Nestler EJ, Hyman SE, Malenka RC (2009b) Excitatory and inhibitory amino acids. In: Molecular Neuropharmacology, 2nd ed. pp 117–144. New York: McGraw-Hill.

Nimchinsky EA, Oberlander AM, Svoboda K (2001) Abnormal development of dendritic spines in FMR1 knock-out mice. J Neurosci 21:5139–5146.

Niswender CM, Conn PJ (2010) Metabotropic glutamate receptors: Physiology, pharmacology, and disease. Annu Rev Pharmacol Toxicol 50:295–322.

Norman RG, Pal I, Stewart C, Walsleben JA, Rapoport DM (2000) Interobserver agreement among sleep scorers from different centers in a large dataset. SLEEP 23:901–908.

Okada T, Mochizuki T, Huang Z-L, Eguchi N, Sugita Y, Urade Y, Hayaishi O (2003) Dominant localization of adenosine deaminase in leptomeninges and involvement of the enzyme in sleep. Biochem Bioph Res Co 312:29–34.

Oliet SH, Malenka RC, Nicoll RA (1997) Two distinct forms of long-term depression coexist in CA1 hippocampal pyramidal cells. Neuron 18:969–982.

Park A, Stacy M (2011) Dopamine-induced nonmotor symptoms of parkinson's disease. Parkinsons Dis 2011:1–6.

Parmentier-Batteur S et al. (2013) Mechanism based neurotoxicity of mGlu5 positive allosteric modulators - Development challenges for a promising novel antipsychotic target. Neuropharmacology :1-13.

Pearson CE, Edamura KN, Cleary JD (2005) Repeat instability: Mechanisms of dynamic mutations. Nat Rev Genet 6:729–742.

Penagarikano O, Mulle JG, Warren ST (2007) The pathophysiology of fragile X syndrome. Annu Rev Genom Human Genet 8:109–129.

Peprah E, He W, Allen E, Oliver T, Boyne A, Sherman SL (2009) Examination of FMR1 transcript and protein levels among 74 premutation carriers. J Hum Genet 55:66–68.

Picó C, Puigserver P, Oliver P, Palou A (1998) 2-Methoxyestradiol, an endogenous metabolite of 17beta-estradiol, inhibits adipocyte proliferation. Mol Cell Biochem 189:1–7.

Popoli P, Pèzzola A, Torvinen M, Reggio R, Pintor A, Scarchilli L, Fuxe K, Ferré S (2001) The selective mGlu5 receptor agonist CHPG inhibits quinpirole-induced turning in 6-Hydroxydopamine-lesioned rats and modulates the binding characteristics of dopamine D2 receptors in the rat striatum. Neuropsychopharmacol 25:505–513.

Porkka-Heiskanen T, Strecker RE, Thakkar M, Bjorkum AA, Greene RW, McCarley RW (1997) Adenosine: A mediator of the sleep-inducing effects of prolonged wakefulness. Science 276:1265–1268.

Portas CM, Rees G, Howseman AM, Josephs O, Turner R, Frith CD (1998) A specific role for the thalamus in mediating the interaction of attention and arousal in humans. J Neurosci 18:8979–8989.

Purves D (2004) Sleep and wakefulness. In: Neuroscience, 3rd ed. pp 659–685. Massachusetts: Sinauer Associates Incorporated.

Qu W-M, Xu X-H, Yan M-M, Wang Y-Q, Urade Y, Huang Z-L (2010) Essential role of dopamine D2 receptor in the maintenance of wakefulness, but not in homeostatic regulation of sleep, in mice. J Neurosci 30:4382–4389.

Ramm P, Smith CT (1990) Rates of cerebral protein synthesis are linked to slow wave sleep in the rat. Physiol Behav 48:749–753.

Rasch B, Born J (2013) About sleep's role in memory. Physiol Rev. 93:681-766

Rasch B, Büchel C, Gais S, Born J (2007) Odor cues during slow-wave sleep prompt declarative memory consolidation. Science 315:1426–1429.

Rebola N, Lujan R, Cunha RA, Mulle C (2008) Adenosine A2A receptors are essential for long-term potentiation of NMDA-EPSCs at hippocampal mossy fiber synapses. Neuron 57:121–134.

Rechtschaffen A, Kales A (1968) A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. Los Angeles: Brain Information Service/Brain Research Institute, University of California.

Rétey JV, Adam M, Gottselig JM, Khatami R, Dürr R, Achermann P, Landolt H-P (2006) Adenosinergic mechanisms contribute to individual differences in sleep deprivation-induced changes in neurobehavioral function and brain rhythmic activity. J Neurosci 26:10472–10479.

Rétey JV, Adam M, Honegger E, Khatami R, Luhmann UFO, Jung HH, Berger W, Landolt H-P (2005) A functional genetic variation of adenosine deaminase affects the duration and intensity of deep sleep in humans. PNAS 102:15676–15681.

Rétey JV, Adam M, Khatami R, Luhmann UFO, Jung HH, Berger W, Landolt H-P (2007) A genetic variation in the adenosine A2A receptor gene (ADORA2A) contributes to individual sensitivity to caffeine effects on sleep. Clin Pharmacol Ther 81:692–698.

Richter CP (1922) A behavioristic study of the activity of the rat. Comparative Psychology Monographs:1–54.

Riedner BA, Hulse BK, Murphy MJ, Ferrarelli F, Tononi G (2011) Temporal dynamics of cortical sources underlying spontaneous and peripherally evoked slow waves. Prog Brain Res 193:201–218.

Robertson KD (2005) DNA methylation and human disease. Nat Rev Genet 6:597–610.

Roediger HL, McDermott KB (1995) Creating false memories: Remembering words not presented in lists. J Exp Psychol Learn Mem Cogn 21:803.

Roenneberg T, Allebrandt KV, Merrow M, Vetter C (2012) Social jetlag and obesity. Curr Biol 22:939–943.

Roenneberg T, Wirz-Justice A, Merrow M (2003) Life between Clocks: Daily Temporal Patterns of Human Chronotypes. J Biol Rhythms 18:80–90.

Romano C, Sesma MA, McDonald CT, O'Malley K, van den Pol AN, Olney JW (1995) Distribution of metabotropic glutamate receptor mGluR5 immunoreactivity in rat brain. J Comp Neurol 355:455–469.

Ronesi JA, Collins KA, Hays SA, Tsai N-P, Guo W, Birnbaum SG, Hu J-H, Worley PF, Gibson JR, Huber KM (2012) Disrupted Homer scaffolds mediate abnormal mGluR5 function in a mouse model of fragile X syndrome. Nature Neurosci 15:431–440.

Ronesi JA, Huber KM (2008) Homer interactions are necessary for metabotropic glutamate receptor-induced long-term depression and translational activation. J Neurosci 28:543–547.

Rousset F (2008) genepop'007: A complete re-implementation of the genepop software for Windows and Linux. Mol Ecol Resour 8:103–106.

Rusterholz T, Dürr R, Achermann P (2010) Inter-individual differences in the dynamics of sleep homeostasis. SLEEP 33:491–498.

Rye DB, Freeman AAH (2011) Dopaminergic substrates underlying hypersomnia, sleepiness, and rEM sleep expression. In: Narcolepsy, 1st ed. (Baumann CR, Bassetti CL, Scammell TE, eds), pp 61–71. New York: Springer.

Rye DB, Jankovic J (2002) Emerging views of dopamine in modulating sleep/wake state from an unlikely source: PD. Neurology 58:341–346.

Sadeh A (2011) The role and validity of actigraphy in sleep medicine: An update. Sleep Med Rev 15:259–267.

Saletu B, Frey R, Krupka M, Anderer P, Grünberger J, Barbanoj MJ (1989) Differential effects of a new central adrenergic agonist--modafinil--and D-amphetamine on sleep and early morning behaviour in young healthy volunteers. Int J Clin Pharmacol Res 9:183–195.

Sanacora G, Zarate CA, Krystal JH, Manji HK (2008) Targeting the glutamatergic system to develop novel, improved therapeutics for mood disorders. Nat Rev Drug Discov 7:426–437.

Sanchez-Vives MV, McCormick DA (2000) Cellular and network mechanisms of rhythmic recurrent activity in neocortex. Nature Neurosci 3:1027–1034.

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. PNAS 74:5463–5467.

Sano H, Yokoi M (2007) Striatal medium spiny neurons terminate in a distinct region in the lateral hypothalamic area and do not directly innervate orexin/hypocretin- or melanin-concentrating hormone-containing neurons. J Neurosci 27:6948–6955.

Saper CB, Chou TC, Scammell TE (2001) The sleep switch: Hypothalamic control of sleep and wakefulness. Trends Neurosci 24:726–731.

Saper CB, Scammell TE, Lu J (2005) Hypothalamic regulation of sleep and circadian rhythms. Nature 437:1257–1263.

Scatton B, Dubois A, Dubocovich ML, Zahniser NR, Fage D (1985) Quantitative autoradiography of 3H-nomifensine binding sites in rat brain. Life Sci 36:815–822.

Schwartz JR, Roth T (2008) Neurophysiology of sleep and wakefulness: basic science and clinical implications. Curr Neuropharmacol 6:367-378.

Schwartz MW, Woods SC, Porte D, Seeley RJ, Baskin DG (2000) Central nervous system control of food intake. Nature 404:661–671.

Sesack SR, Hawrylak VA, Matus C, Guido MA, Levey AI (1998) Dopamine axon varicosities in the prelimbic division of the rat prefrontal cortex exhibit sparse immunoreactivity for the dopamine transporter. J Neurosci 18:2697–2708.

Sherin JE, Shiromani PJ, McCarley RW, Saper CB (1996) Activation of ventrolateral preoptic neurons during sleep. Science 271:216–219.

Sobanski E, Schredl M, Kettler N, Alm B (2008) Sleep in adults with attention deficit hyperactivity disorder (ADHD) before and during treatment with methylphenidate: A controlled polysomnographic study. SLEEP 31:375–381.

Sonders MS, Zhu SJ, Zahniser NR, Kavanaugh MP, Amara SG (1997) Multiple ionic conductances of the human dopamine transporter: the actions of dopamine and psychostimulants. J Neurosci 17:960–974.

Spencer TJ, Biederman J, Faraone SV, Madras BK, Bonab AA, Dougherty DD, Batchelder H, Clarke A, Fischman AJ (2013) Functional genomics of attention-deficit/hyperactivity disorder (ADHD) risk alleles on dopamine transporter binding in ADHD and healthy control subjects. Biol Psychiat 74:84–89.

Spencer TJ, Biederman J, Madras BK, Faraone SV, Dougherty DD, Bonab AA, Fischman AJ (2005) In vivo neuroreceptor imaging in attention-deficit/hyperactivity disorder: A focus on the dopamine transporter. Biol Psychiat 57:1293–1300.

Spielberger CD, Gorsuch RL, Lushene RE (1970) Manual for the State-Trait Anxiety Inventory. Palo Alto, CA: Consulting Psychologist Press.

Steinfels GF, Heym J, Strecker RE, Jacobs BL (1983) Behavioral correlates of dopaminergic unit activity in freely moving cats. Brain Res 258:217–228.

Stephan FK, Zucker I (1972) Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. PNAS 69:1583–1586.

Steriade M (1970) Ascending control of thalamic and cortical responsiveness. Int Rev Neurobiol 12:87–144.

Steriade M, Datta S, Paré D, Oakson G, Curró Dossi RC (1990a) Neuronal activities in brain-stem cholinergic nuclei related to tonic activation processes in thalamocortical systems. J Neurosci 10:2541–2559.

Steriade M, Dossi RC, Nuñez A (1991) Network modulation of a slow intrinsic oscillation of cat thalamocortical neurons implicated in sleep delta waves: cortically induced synchronization and brainstem cholinergic suppression. J Neurosci 11:3200–3217.

Steriade M, Jones EG, Llinás RR (1990b) Thalamic oscillations and signaling, 1st ed. New York: Wiley-Interscience.

Steriade M, Nuñez A, Amzica F (1993a) A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. J Neurosci 13:3252–3265.

Steriade M, Nuñez A, Amzica F (1993b) Intracellular analysis of relations between the slow (< 1 Hz) neocortical oscillation and other sleep rhythms of the electroencephalogram. J Neurosci 13:3266–3283.

Steriade M, Timofeev I (2003) Neuronal plasticity in thalamocortical networks during sleep and waking oscillations. Neuron 37:563–576.

Surman CBH, Adamson JJ, Petty C, Biederman J, Kenealy DC, Levine M, Mick E, Faraone SV (2009) Association between attention-deficit/hyperactivity disorder and sleep impairment in adulthood: Evidence from a large controlled study. J Clin Psychiatry 70:1523–1529.

Swanson CJ, Bures M, Johnson MP, Linden A-M, Monn JA, Schoepp DD (2005) Metabotropic glutamate receptors as novel targets for anxiety and stress disorders. Nat Rev Drug Discov 4:131–144.

Swanson JM, Kinsbourne M, Nigg J, Lanphear B, Stefanatos GA, Volkow N, Taylor E, Casey BJ, Castellanos FX, Wadhwa PD (2007) Etiologic subtypes of attention-deficit/hyperactivity disorder: Brain imaging, molecular genetic and environmental factors and the dopamine hypothesis. Neuropsychol Rev 17:39–59.

Tadavarty R, Rajput PS, Wong JM, Kumar U, Sastry BR (2011) Sleep-deprivation induces changes in GABAB and mGlu receptor expression and has consequences for synaptic long-term depression. PLoS ONE 6:e24933.

Takahashi K, Kayama Y, Lin JS, Sakai K (2010) Locus coeruleus neuronal activity during the sleep-waking cycle in mice. Neuroscience 169:1115–1126.

Takahashi K, Lin J-S, Sakai K (2006) Neuronal activity of histaminergic tuberomammillary neurons during wake-sleep states in the mouse. J Neurosci 26:10292–10298.

Takashima A, Petersson KM, Rutters F, Tendolkar I, Jensen O, Zwarts MJ, McNaughton BL, Fernandez G (2006) Declarative memory consolidation in humans: a prospective functional magnetic resonance imaging study. PNAS 103:756–761.

Tassone F, Hagerman RJ, Taylor AK, Gane LW, Godfrey TE, Hagerman PJ (2000) Elevated levels of FMR1 mRNA in carrier males: A new mechanism of involvement in the fragile-X syndrome. Am J Hum Genet 66:6–15.

Tebano MT, Martire A, Rebola N, Pepponi R, Domenici MR, Gro MC, Schwarzschild MA, Chen JF, Cunha RA, Popoli P (2005) Adenosine A2A receptors and metabotropic glutamate 5 receptors are co-localized and functionally interact in the hippocampus: A possible key mechanism in the

modulation of N-methyl-d-aspartate effects. J Neurochem 95:1188–1200.

Timofeev I, Steriade M (1996) Low-frequency rhythms in the thalamus of intact-cortex and decorticated cats. J Neurophysiol 76:4152–4168.

Tobler I (2011) Phylogeny of Sleep Regulation. In: Principles and Practice of Sleep Medicine, 5 ed. Philadelphia: Saunders.

Tonetti L, Pasquini F, Fabbri M, Belluzzi M, Natale V (2008) Comparison of two different actigraphs with polysomnography in healthy young subjects. Chronobiol Int 25:145–153.

Tononi G, Cirelli C (2003) Sleep and synaptic homeostasis: A hypothesis. Brain Res Bull 62:143–150.

Tononi G, Cirelli C (2006) Sleep function and synaptic homeostasis. Sleep Med Rev 10:49–62.

Tononi G, Cirelli C (2012) Time to be SHY? Some comments on sleep and synaptic homeostasis. Neural Plast 2012:415250.

Torres GE, Gainetdinov RR, Caron MG (2003) Plasma membrane monoamine transporters: Structure, regulation and function. Nat Rev Neurosci 4:13–25.

Trulson ME, Preussler DW (1984) Dopamine-containing ventral tegmental area neurons in freely moving cats: activity during the sleep-waking cycle and effects of stress. Exp Neurol 83:367-77.

Tunbridge EM, Harrison PJ, Weinberger DR (2006) Catechol-o-methyltransferase, cognition, and psychosis: Val158Met and beyond. Biol Psychiat 60:141–151.

Tworoger SS, Chubak J, Aiello EJ, Yasui Y, Ulrich CM, Farin FM, Stapleton PL, Irwin ML, Potter JD, Schwartz RS, McTiernan A (2004) The effect of CYP19 and COMT polymorphisms on exercise-induced fat loss in postmenopausal women. Obes Res 12:972–981.

Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Mazoyer B, Joliot M (2002) Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. Neuroimage 15:273–289.

Ueno T, Tomita J, Tanimoto H, Endo K, Ito K, Kume S, Kume K (2012) Identification of a dopamine pathway that regulates sleep and arousal in Drosophila. Nature Neurosci 15:1516–1523.

Urade Y, Eguchi N, Qu W-M, Sakata M, Huang Z-L, Chen J-F, Schwarzschild MA, Fink JS, Hayaishi O (2003) Sleep regulation in adenosine A2A receptor-deficient mice. Neurology 61:S94–S96.

Usuda I, Tanaka K, Chiba T (1998) Efferent projections of the nucleus accumbens in the rat with special reference to subdivision of the nucleus: biotinylated dextran amine study. Brain Res 797:73–93.

Van Beijsterveldt CE, Molenaar PC, De Geus EJ, Boomsma DI (1996) Heritability of human brain functioning as assessed by electroencephalography. Am J Hum Genet 58:562.

van de Giessen EM, de Win MML, Tanck MWT, van den Brink W, Baas F, Booij J (2009) Striatal dopamine transporter availability associated with polymorphisms in the dopamine transporter gene SLC6A3. J Nucl Med 50:45–52.

van den Pol AN, Romano C, Ghosh P (1995) Metabotropic glutamate receptor mGluR5 subcellular distribution and developmental expression in hypothalamus. J Comp Neurol 362:134–150.

Van Dongen HP, Dinges DF (2003) Investigating the interaction between the homeostatic and circadian processes of sleep–wake regulation for the prediction of waking neurobehavioural performance. J Sleep Res 12:181–187.

van Dyck CH, Malison RT, Jacobsen LK, Seibyl JP, Staley JK, Laruelle M, Baldwin RM, Innis RB, Gelernter J (2005) Increased dopamine transporter availability associated with the 9-repeat allele of the SLC6A3 gene. J Nucl Med 46:745–751.

van Hilten B, Hoff JI, Middelkoop HA, van der Velde EA, Kerkhof GA, Wauquier A, Kamphuisen HA, Roos RA (1994) Sleep disruption in Parkinson's disease. Assessment by continuous activity

monitoring. Arch Neurol 51:922–928.

Van Someren EJ, Swaab DF, Colenda CC, Cohen W, McCall WV, Rosenquist PB (1999) Bright light therapy: improved sensitivity to its effects on rest-activity rhythms in Alzheimer patients by application of nonparametric methods. Chronobiol Int 16:505–518.

Van Veen MM, Kooij JJS, Boonstra AM, Gordijn MCM, van Someren EJW (2010) Delayed circadian rhythm in adults with attention-deficit/hyperactivity disorder and chronic sleep-onset insomnia. Biol Psychiat 67:1091–1096.

Vandenbergh DJ, Persico AM, Hawkins AL, Griffin CA, Li X, Jabs EW, Uhl GR (1992) Human dopamine transporter gene (DAT1) maps to chromosome 5p15.3 and displays a VNTR. Genomics 14:1104–1106.

VanNess SH, Owens MJ, Kilts CD (2005) The variable number of tandem repeats element in DAT1 regulates in vitro dopamine transporter density. BMC Genet 6:55.

Viola AU, Archer SN, James LM, Groeger JA, Lo JCY, Skene DJ, Schantz von M, Dijk D-J (2007) PER3 polymorphism predicts sleep structure and waking performance. Curr Biol 17:613–618.

Viwatpinyo K, Chongthammakun S (2009) Activation of group I metabotropic glutamate receptors leads to brain-derived neurotrophic factor expression in rat C6 cells. Neurosci Lett 467:127–130.

Volkow ND, Fowler JS, Logan J, Alexoff D, Zhu W, Telang F, Wang G-J, Jayne M, Hooker JM, Wong C, Hubbard B, Carter P, Warner D, King P, Shea C, Xu Y, Muench L, Apelskog-Torres K (2009a) Effects of modafinil on dopamine and dopamine transporters in the male human brain. JAMA 301:1148–1154.

Volkow ND, Tomasi D, Wang G-J, Telang F, Fowler JS, Wang RL, Logan J, Wong C, Jayne M, Swanson JM (2009b) Hyperstimulation of striatal D2 receptors with sleep deprivation: Implications for cognitive impairment. Neuroimage 45:1232–1240.

Volkow ND, Tomasi D, Wang GJ, Telang F, Fowler JS, Logan J, Benveniste H, Kim R, Thanos PK, Ferré S (2012) Evidence That Sleep Deprivation Downregulates Dopamine D2R in Ventral Striatum in the Human Brain. J Neurosci 32:6711–6717.

Volkow ND, Wang G-J, Telang F, Fowler JS, Logan J, Wong C, Ma J, Pradhan K, Tomasi D, Thanos PK, Ferré S, Jayne M (2008) Sleep deprivation decreases binding of [11C]raclopride to dopamine D2/D3 receptors in the human brain. J Neurosci 28:8454–8461.

Volkow ND, Wang GJ, Fowler JS, Gatley SJ, Logan J, Ding YS, Hitzemann R, Pappas N (1998) Dopamine transporter occupancies in the human brain induced by therapeutic doses of oral methylphenidate. Am J Psychiat 155:1325–1331.

Vyazovskiy VV, Cirelli C, Pfister-Genskow M, Faraguna U, Tononi G (2008a) Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. Nature Neurosci 11:200–208.

Vyazovskiy VV, Cirelli C, Tononi G, Tobler I (2008b) Cortical metabolic rates as measured by 2-deoxyglucose-uptake are increased after waking and decreased after sleep in mice. Brain Res Bull 75:591–597.

Vyazovskiy VV, Olcese U, Lazimy YM, Faraguna U, Esser SK, Williams JC, Cirelli C, Tononi G (2009) Cortical firing and sleep homeostasis. Neuron 63:865–878.

Wang GJ, Volkow ND, Logan J, Pappas NR, Wong CT, Zhu W, Netusil N, Fowler JS (2001) Brain dopamine and obesity. Lancet 357:354–357.

Wesensten NJ (2006) Effects of modafinil on cognitive performance and alertness during sleep deprivation. Curr Pharm Des 12:2457–2471.

Westbrook GL (2000) Seizures and Epilepsy. In: Principles of Neural Science, 4 ed. pp 912–935. New York: McGraw-Hill Professional.

Weyers P, Krebs H, Janke W (1995) Reliability and construct validity of the German version of Cloninger's Tridimensional Personality Questionnaire. Pers Indiv Differ 19:853–861.

Whitehead DL, Davies ADM, Playfer JR, Turnbull CJ (2008) Circadian rest-activity rhythm is altered in Parkinson's disease patients with hallucinations. Mov Disorders 23:1137–1145.

Wilkening S, Chen B, Bermejo JL, Canzian F (2009) Is there still a need for candidate gene approaches in the era of genome-wide association studies? Genomics 93:415–419.

Wise RA, Bozarth MA (1987) A psychomotor stimulant theory of addiction. Psychol Rev 94:469–492.

Wisor JP, Nishino S, Sora I, Uhl GH, Mignot E, Edgar DM (2001) Dopaminergic role in stimulant-induced wakefulness. J Neurosci 21:1787–1794.

Wu MN, Koh K, Yue Z, Joiner WJ, Sehgal A (2008) A genetic screen for sleep and circadian mutants reveals mechanisms underlying regulation of sleep in Drosophila. SLEEP 31:465–472.

Xu J, Zhu Y, Contractor A, Heinemann SF (2009) mGluR5 has a critical role in inhibitory learning. J Neurosci 29:3676–3684.

Yoshida K, McCormack S, España RA, Crocker A, Scammell TE (2006) Afferents to the orexin neurons of the rat brain. J Comp Neurol 494:845–861.

Youdim MBH, Edmondson D, Tipton KF (2006) The therapeutic potential of monoamine oxidase inhibitors. Nat Rev Neurosci 7:295–309.

Yrigollen CM, Durbin-Johnson B, Gane L, Nelson DL, Hagerman RJ, Hagerman PJ, Tassone F (2012) AGG interruptions within the maternal FMR1 gene reduce the risk of offspring with fragile X syndrome. Genet Med:729–736.

Yrigollen CM, Tassone F, Durbin-Johnson B, Tassone F (2011) The Role of AGG Interruptions in the Transcription of FMR1 Premutation Alleles Weedon MN, ed. PLoS ONE 6:e21728.

Zhu M, Zhao S (2007) Candidate gene identification approach: progress and challenges. Int J Biol Sci 3:420–427.

Zumwalt M, Ludwig A, Hagerman PJ, Dieckmann T (2007) Secondary structure and dynamics of the r(CGG) repeat in the mRNA of the fragile X mental retardation 1 (FMR1) gene. RNA Biol 4:93–100.

Appendix 1

Supplementary information to Chapter 3

Dopaminergic role in regulating neurophysiological markers of sleep homeostasis in humans

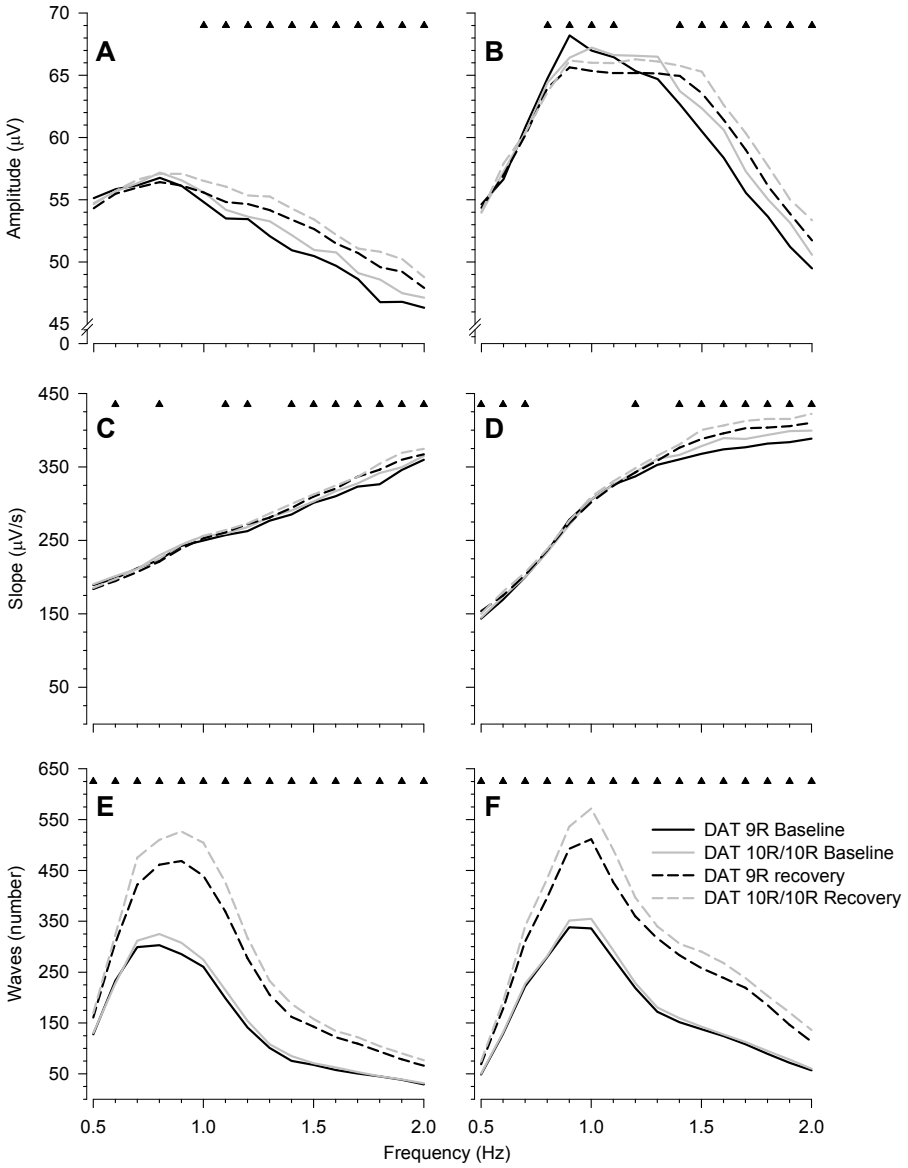
Supplementary Table 1: Visually scored sleep variables in 10R/10R (n = 30) and 9R allele carriers (n = 27) of *DAT1*.

	10R/10R		9R allele carriers		*'genotype'*	*'condition'*	*'genotype'* x *'condition'*
	Baseline	Recovery	Baseline	Recovery	$F_{1,55}$, *p*	$F_{1,55}$, *p*	$F_{1,55}$, *p*
Sleep efficiency (%)	93.4 ± 0.7	97.2 ± 0.2	93.1 ± 0.7	97.1 ± 0.2	0.2, 0.67	61.8, <0.0001	0.1, 0.76
Stage 1 (min)	37.0 ± 2.5	18.9 ± 2.3	33.0 ± 2.4	16.6 ± 1.9	1.1, 0.30	163.0, <0.0001	0.4, 0.55
Stage 2 (min)	221.0 ± 6.4	200.0 ± 7.0	213.0 ± 5.3	203.0 ± 7.7	0.1, 0.77	21.3, <0.0001	3.0, 0.09
SWS (min)	93.6 ± 6.5	158.0 ± 7.1	100.0 ± 5.4	149.0 ± 6.8	0.0, 0.91	639.9, <0.0001	11.0, 0.002
NREM sleep (min)	315.0 ± 4.0	357.0 ± 4.6	313.0 ± 4.0	352.0 ± 5.7	0.4, 0.53	150.1, <0.0001	0.2, 0.64
REM sleep (min)	96.4 ± 3.3	90.2 ± 4.5	101.0 ± 3.3	97.3 ± 5.3	1.2, 0.27	2.5, 0.12	0.2, 0.63
MT (min)	9.4 ± 0.8	9.7 ± 0.8	10.7 ± 0.8	9.3 ± 0.6	0.2, 0.65	1.6, 0.21	3.2, 0.08
WASO (min)	9.0 ± 2.6	0.8 ± 0.4	5.3 ± 1.3	0.9 ± 0.3	1.4, 0.25	17.3, 0.0001	1.6, 0.22
Sleep latency (min)	13.1 ± 2.1	3.1 ± 0.4	17.2 ± 3.1	3.8 ± 0.5	1.6, 0.22	42.6, <0.0001	0.9, 0.34
REM latency (min)	70.1 ± 4.1	83.2 ± 7.8	64.6 ± 3.0	70.9 ± 3.5	0.2, 0.67	61.8, <0.0001	0.1, 0.76

Values represent means ± SEM in baseline and recovery nights. Analyses of the recovery nights were restricted to 480 minutes. Sleep efficiency: percentage of total sleep time per 480 minutes. Stages 1, stage 2 and SWS: non-rapid-eye-movement (NREM) sleep stages. REM sleep: rapid-eye-movement sleep. MT: movement time. WASO: wakefulness after sleep onset. Sleep latency: time from lights-out to the first occurrence of stage 2 sleep. REM latency: time from sleep onset to the first occurrence of REM sleep.

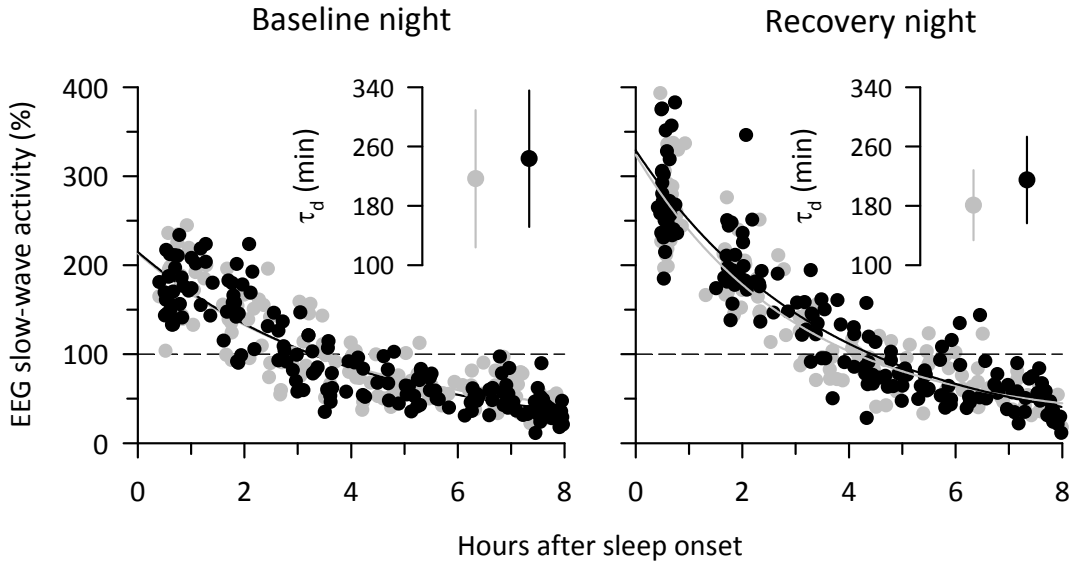
F- and p-values: Two-way mixed-model ANOVA with factors *'genotype'* (10R/10R, 9R) and *'condition'* (baseline, recovery).

Supplementary Figure 1. Prolonged wakefulness increases number, amplitude, and slope of EEG slow waves in NREM sleep



Effect of sleep deprivation on amplitude (**A** & **B**), slope (**C** & **D**) and number (**E** & **F**) of positive **(left)** and negative **(right)** slow (0.5-2.0 Hz) half-waves in NREM sleep in baseline (continuous lines) and recovery nights (dotted lines) in 10R/10R (gray, n = 30) and 9R (black, n = 27) genotypes of DAT1. Except for negative half-waves between 0.8-1.1 Hz, sleep deprivation increased amplitude, slope and number of half-waves (three-way mixed model ANOVA 'condition': $F_{1,55} > 93.7$, $p_{all} < 0.0001$). The sleep deprivation-induced increase in the number of negative and positive slow half was significantly larger in 10R/10R carriers than in 9R carriers of DAT1. Three-way mixed model ANOVA: 'condition': $F_{1,55} \geq 3977.0$, $p \leq 0.0001$; 'genotype': ns; 'frequency bin': $F_{15,825} \geq 55.3$, $p \leq 0.0001$; 'genotype' × 'deprivation': $F_{1,55} \geq 40.6$, $p \leq 0.0001$; 'condition' × 'frequency bins': $F_{15,840} \geq 35.9$, $p \leq 0.0001$; 'genotype' × 'frequency bins': ns. Triangles on top of the panels indicate frequency bins that were significantly affected by sleep deprivation ($p < 0.05$, 2-tailed paired t-tests).

Supplementary Figure S2. Exponential decay of homeostatically regulated EEG slow wave activity across consecutive NREM sleep episodes in 10R/10R and 9R allele carriers of DAT1



Exponential decay of EEG slow-wave activity (SWA, 0.5-4.5 Hz) in baseline (**left**) and recovery (**right**) nights. Individual SWA values per NREM sleep episode, expressed as a percentage of the corresponding all-night value in baseline (horizontal dashed line at 100 %), were plotted at episode midpoint relative to sleep onset. The lines represent exponential decay fits of the homeostatic process S, in 10R/10R (black dots, black line) and 9R (grey dots, grey line) carriers. Insets: Time constants (τ_d ± 95% confidence interval) represent the best exponential decay fits. Overlapping confidence intervals suggest that τ_d did not differ between 10R/10R and 9R genotypes.

Modeling of the homeostatic Process S was performed as described by (Rusterholz et al., 2010). To summarize, individual SWA (0.5-4.5 Hz) in NREM sleep was normalized to whole-night baseline levels and plotted against individual NREM sleep episode midpoints. Decreasing exponential functions were fitted to mean episodic SWA values using the function:

$$S(t) = (S_0 - LA) \cdot \exp\left(-\frac{t}{\tau_d}\right) + LA$$

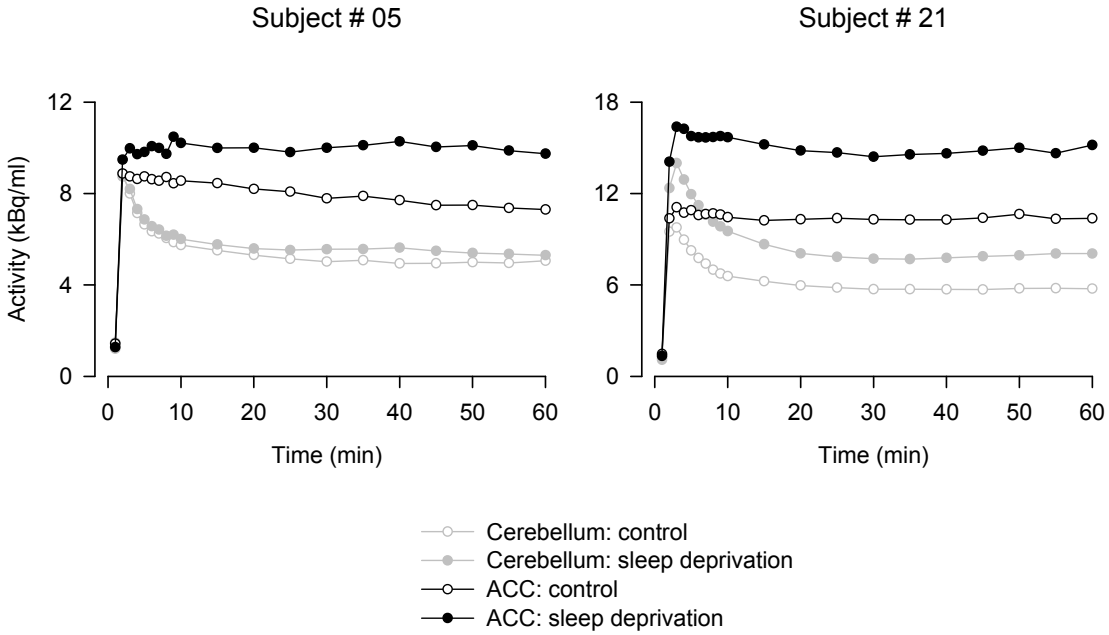
Where S(t) is the time dependent homeostatic process, S_0 is the level of S at sleep onset, *LA* is the lower asymptote required to be positive whereas the time constant, τ_d, reflects the dissipation of sleep pressure during sleep. To investigate effects of the DAT1 polymorphism, the fit was done separately in 10R/10R and 9R allele carriers in baseline and recovery nights.

Appendix 2

Supplementary information to chapter 4

Increased metabotropic glutamate receptor subtype 5 availability in human brain after one night without sleep

Figure S1. Tissue time-activity-curves (TAC)



Tissue time-activity-curves (TAC) of cerebellum (grey symbols) and anterior cingulate cortex (ACC; black symbols) of representative subjects # 05 and 21 show that in sleep control (open symbols) and sleep deprivation (filled symbols) conditions a steady state of receptor binding was reached after 45-55 min of image acquisition. The AAC was chosen because this region showed the highest ^{11}C-ABP688 binding in both conditions.

Sleep deprivation induced no change in cerebellar V_{ND}

To address the question whether sleep deprivation changed ^{11}C-ABP688 binding in the cerebellum, which could have driven the results in the other regions, we calculated the non-displaceable volume of distribution (V_{ND}) in sleep control and sleep deprivation conditions in those 9 study participants in whom reliable blood metabolite concentrations were available. These analyses confirmed that ^{11}C-ABP688 binding in the cerebellum was not changed after sleep deprivation when compared to the control condition.

$V_{ND} = C_{Cb}/C_{plasma}$, where C_{plasma} is the ^{11}C-ABP688 concentration (kBq/mL) in plasma.

Sleep control: $V_{ND} = 1.98 \pm 0.14$

Sleep deprivation: $V_{ND} = 2.18 \pm 0.16$ $p = 0.19$ (2-tailed, paired t-test, n = 9)

Table S1: Individual normalized volumes of distribution (V_{norm}) in sleep control and sleep deprivation conditions.

#	Ant. cingulate cortex		Insula		Medial temporal lobe		Orbitofrontal cortex		Parahippo-campal gyrus		Med. sup. frontal gyrus		Dorsolat. pre-frontal cortex		Striatum		Precuneus		Parietal cortex		Amygdala		Hippocampus		Thalamus	
	Ctrl.	SD	Ctrl.	SD	Ctrl.	SD	Ctrl.	SD	Ctrl.	SD	Ctrl.	SD	Ctrl.	SD	Ctrl.	SD	Ctrl.	SD	Ctrl.	SD	Ctrl.	SD	Ctrl.	SD	Ctrl.	SD
2	2.044	1.995	1.882	1.880	1.769	1.781	1.728	1.754	1.777	1.814	1.759	1.793	1.598	1.643	1.561	1.655	1.556	1.542	1.537	1.524	1.656	1.640	1.319	1.369	1.248	1.305
3	1.778	1.861	1.710	1.785	1.620	1.698	1.605	1.655	1.550	1.639	1.605	1.639	1.503	1.557	1.460	1.495	1.477	1.506	1.447	1.519	1.420	1.454	1.218	1.243	1.186	1.177
4	1.692	1.854	1.593	1.747	1.581	1.731	1.531	1.651	1.488	1.654	1.427	1.546	1.374	1.500	1.404	1.520	1.329	1.429	1.319	1.446	1.425	1.597	1.228	1.347	1.201	1.280
5	1.648	1.726	1.526	1.640	1.523	1.667	1.528	1.617	1.482	1.604	1.455	1.516	1.365	1.427	1.314	1.380	1.338	1.417	1.342	1.433	1.398	1.478	1.203	1.253	1.173	1.224
6	1.790	1.816	1.760	1.794	1.627	1.657	1.622	1.650	1.672	1.692	1.574	1.585	1.485	1.510	1.514	1.542	1.484	1.467	1.448	1.436	1.530	1.487	1.368	1.331	1.255	1.281
7	1.542	1.731	1.460	1.608	1.405	1.502	1.382	1.529	1.460	1.525	1.370	1.503	1.274	1.381	1.291	1.418	1.268	1.327	1.248	1.327	1.276	1.340	1.129	1.141	1.175	1.218
8	1.618	1.831	1.491	1.652	1.475	1.633	1.447	1.581	1.378	1.571	1.453	1.582	1.372	1.488	1.314	1.447	1.308	1.383	1.306	1.412	1.276	1.362	1.072	1.161	1.170	1.217
10	1.813	1.787	1.750	1.745	1.615	1.635	1.624	1.617	1.530	1.576	1.579	1.589	1.494	1.487	1.404	1.389	1.451	1.438	1.479	1.456	1.398	1.440	1.144	1.138	1.127	1.092
11	1.786	1.698	1.635	1.598	1.651	1.591	1.628	1.546	1.555	1.508	1.525	1.508	1.420	1.400	1.312	1.340	1.477	1.440	1.515	1.487	1.428	1.415	1.212	1.217	1.187	1.169
12	1.799	1.914	1.798	1.892	1.686	1.753	1.649	1.701	1.606	1.676	1.602	1.679	1.554	1.631	1.624	1.685	1.532	1.573	1.494	1.489	1.385	1.496	1.324	1.362	1.270	1.292
13	1.647	1.705	1.582	1.635	1.517	1.545	1.485	1.530	1.376	1.429	1.432	1.481	1.395	1.432	1.362	1.404	1.350	1.378	1.390	1.425	1.287	1.362	1.081	1.119	1.009	1.049
14	1.666	1.500	1.559	1.455	1.470	1.413	1.462	1.357	1.490	1.402	1.419	1.320	1.358	1.246	1.372	1.270	1.286	1.237	1.285	1.249	1.321	1.263	1.164	1.104	1.134	1.053
15	1.915	2.118	1.731	1.939	1.678	1.820	1.657	1.773	1.622	1.805	1.603	1.821	1.531	1.701	1.579	1.855	1.440	1.576	1.453	1.520	1.475	1.701	1.270	1.574	1.319	1.444
16	1.586	1.739	1.486	1.636	1.494	1.617	1.453	1.561	1.387	1.523	1.340	1.436	1.316	1.403	1.264	1.403	1.324	1.417	1.354	1.441	1.343	1.434	1.110	1.205	1.095	1.201
17	1.926	1.893	1.872	1.849	1.762	1.739	1.769	1.741	1.636	1.664	1.701	1.602	1.681	1.532	1.637	1.617	1.568	1.437	1.489	1.413	1.491	1.490	1.354	1.325	1.336	1.282
18	1.969	1.976	1.946	1.969	1.838	1.858	1.780	1.798	1.687	1.719	1.757	1.776	1.659	1.675	1.660	1.686	1.642	1.673	1.577	1.579	1.559	1.593	1.339	1.348	1.289	1.285
19	2.014	1.972	1.850	1.751	1.786	1.794	1.754	1.697	1.760	1.700	1.772	1.653	1.660	1.558	1.737	1.605	1.608	1.568	1.605	1.568	1.605	1.559	1.386	1.294	1.370	1.305
20	1.730	1.906	1.568	1.786	1.540	1.707	1.542	1.687	1.491	1.665	1.546	1.695	1.461	1.603	1.438	1.650	1.465	1.596	1.526	1.637	1.422	1.596	1.211	1.363	1.265	1.400
21	1.771	1.894	1.604	1.770	1.594	1.690	1.592	1.686	1.617	1.726	1.484	1.645	1.411	1.535	1.398	1.569	1.316	1.404	1.358	1.451	1.399	1.530	1.215	1.369	1.217	1.287
22	1.728	1.832	1.668	1.722	1.617	1.680	1.612	1.669	1.517	1.567	1.614	1.670	1.516	1.576	1.443	1.479	1.517	1.532	1.467	1.535	1.460	1.502	1.220	1.266	1.224	1.217
23	1.593	1.664	1.634	1.751	1.573	1.680	1.514	1.626	1.523	1.593	1.408	1.484	1.370	1.455	1.376	1.470	1.339	1.404	1.374	1.432	1.374	1.444	1.191	1.284	1.181	1.249
mean	1.765	1.829	1.672	1.743	1.611	1.676	1.589	1.639	1.553	1.621	1.544	1.596	1.467	1.511	1.451	1.518	1.432	1.464	1.429	1.466	1.425	1.485	1.227	1.277	1.211	1.239
STD	0.144	0.137	0.142	0.125	0.114	0.106	0.112	0.101	0.114	0.109	0.130	0.124	0.117	0.110	0.135	0.142	0.114	0.102	0.100	0.085	0.102	0.107	0.094	0.112	0.084	0.098
	*		*		*				*						*						*					

Values represent cerebellum-normalized volumes of distribution (V_{norm}) of ^{11}C-ABP688 uptake in 13 volumes-of-interest (VOI) after 9 (control, Ctrl.) and 32 hours (sleep deprivation, SD) of wakefulness. Mean values + SEM are also presented in Fig. 2. Asterisks indicate those six VOI that showed a significant increase in V_{norm} after sleep deprivation (Bonferroni-corrected p-value of factor 'condition': $p < 0.0038$). Ant. cingulate cortex = anterior cingulate cortex; Med. sup. frontal cortex = medial superior frontal cortex; Dorssolat. prefrontal cortex = dorsolateral prefrontal cortex; STD= standard deviation.

Appendix 3

Supplementary information to chapter 5

Human electroencephalographic markers of sleep need is associated with cerebral mGluR5 availability and modulated by the fragile X gene

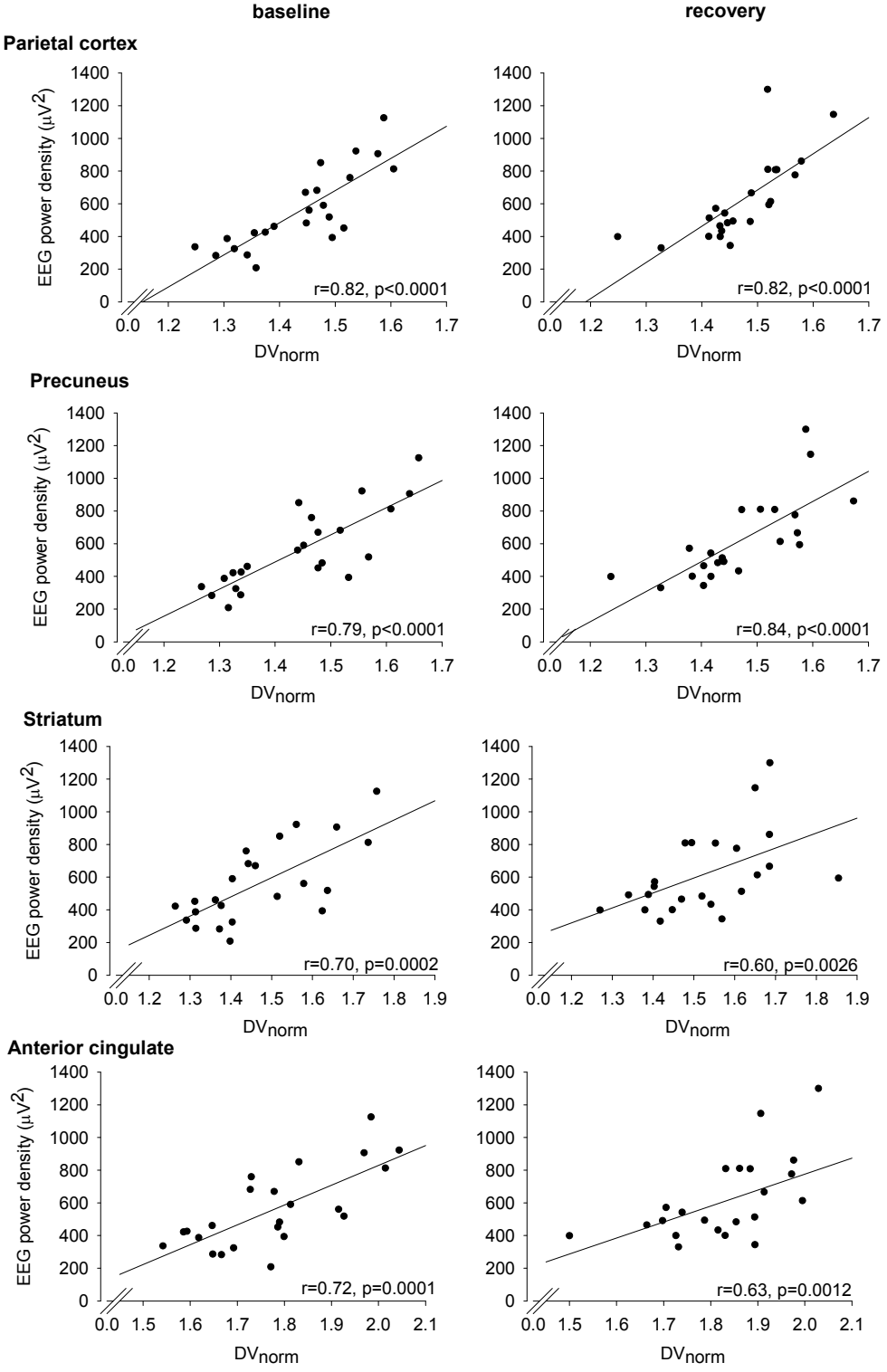
Additional Table 1: Effects of sleep deprivation on sleep stages in relation to the FMR1 odd vs even genotype

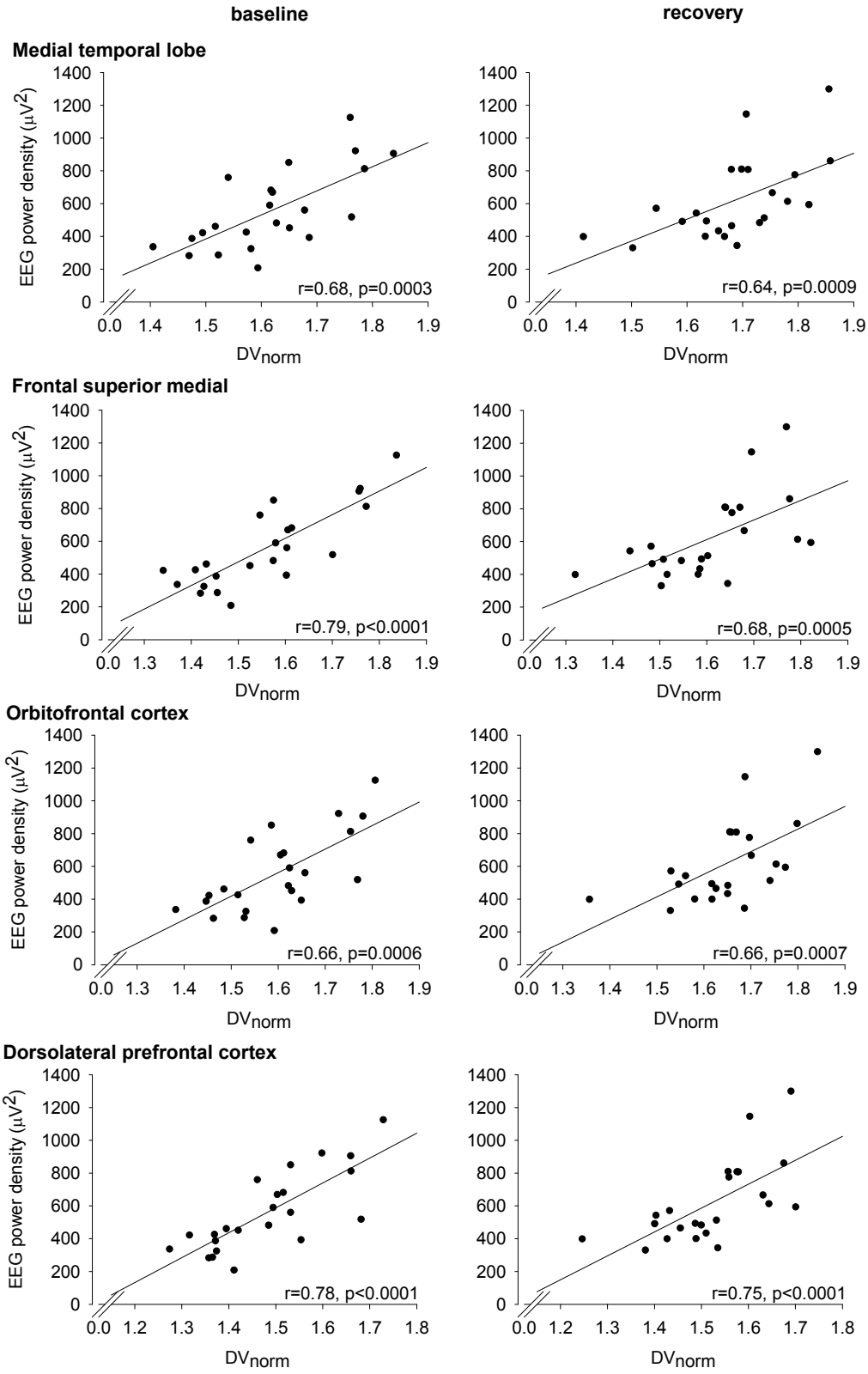
	FMR1 odd (n=12)		*FMR1* even (n=14)		Two-way ANOVA (*p*-values)		
	Baseline	Recovery	Baseline	Recovery	'condition'	'genotype'	'condition × genotype'
Sleep efficiency (%)	93.7 ± 0.96	97.6 ± 0.41	95.4 ± 0.48	98.3 ± 0.25	**<0.0001**	**<0.05**	0.32
Stage 1 (min)	24.2 ± 3.60	13.2 ± 2.62	18.4 ± 2.92	7.19 ± 1.00	**<0.0004**	**<0.04**	0.97
Stage 2 (min)	218 ± 7.19	203 ± 8.94	229 ± 4.82	212 ± 7.31	**<0.05**	0.17	0.86
SWS (min)	97.4 ± 6.52	140 ± 6.71	89.7 ± 6.32	143 ± 8.44	**<0.0001**	0.73	0.48
NREM sleep (min)	315 ± 5.86	343 ± 5.78	319 ± 6.44	355 ± 5.97	**<0.0001**	0.22	0.52
REM sleep (min)	110 ± 5.66	112 ± 3.88	120 ± 4.47	109 ± 5.74	0.37	0.46	0.23
MT (min)	6.31 ± 0.94	6.53 ± 1.46	5.83 ± 0.66	4.79 ± 0.63	0.67	0.25	0.51
WASO (min)	5.89 ± 2.46	1.47 ± 0.53	3.90 ± 1.26	0.74 ± 0.42	**<0.02**	0.33	0.65
Sleep latency (min)	16.1 ± 3.22	3.36 ± 0.73	12.2 ± 1.43	2.79 ± 0.73	**<0.0001**	0.21	0.35
REM latency (min)	60.7 ± 2.97	58.4 ± 3.65	65.3 ± 3.79	64.6 ± 3.90	0.69	0.15	0.84

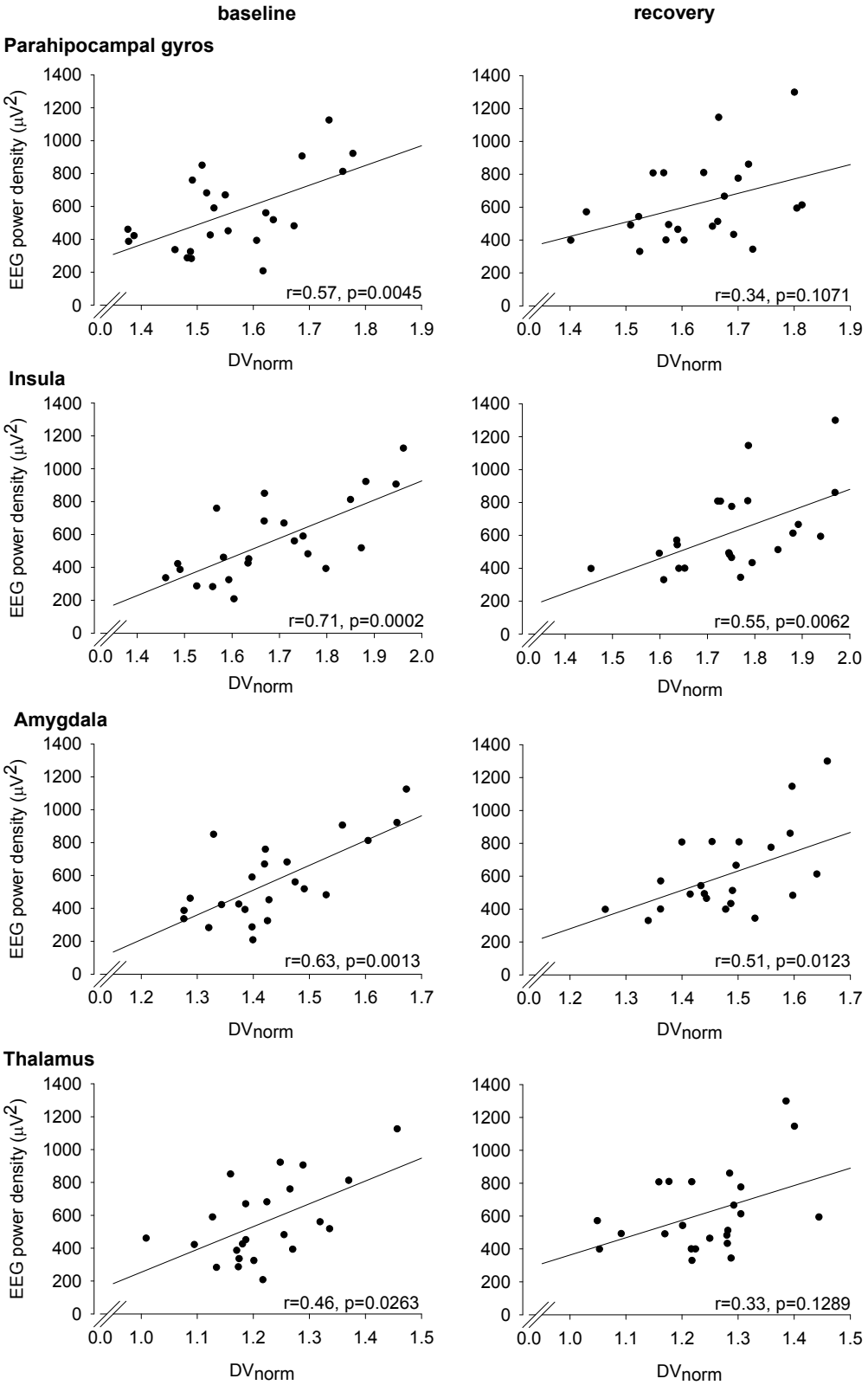
Values represent means ± SEM in baseline and recovery nights. Analyses of the recovery nights were restricted to 480 minutes. Sleep efficiency: percentage of total sleep time per 480 minutes. Stages 1, stage 2 and SWS: non-rapid-eye-movement (NREM) sleep stages. REM sleep: rapid-eye-movement sleep. MT: movement time. WASO: wakefulness after sleep onset. Sleep latency: time from lights-out to the first occurrence of stage 2 sleep. REM latency: time from sleep onset to the first occurrence of REM sleep.

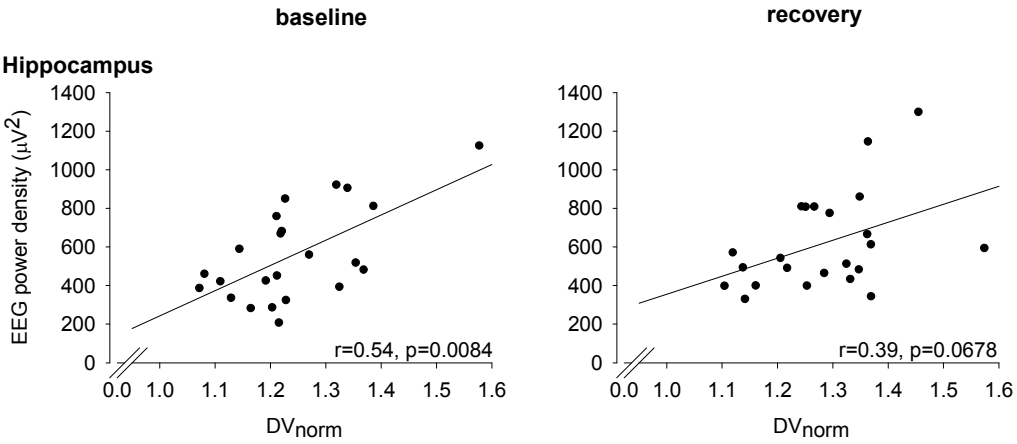
p-values: Two-way mixed-model ANOVA with factors *'genotype'* (odd, even) and *'condition'* (baseline, recovery).

Supplementary figure 1. Regional specific correlations between mGluR5 availability and EEG slow oscillations



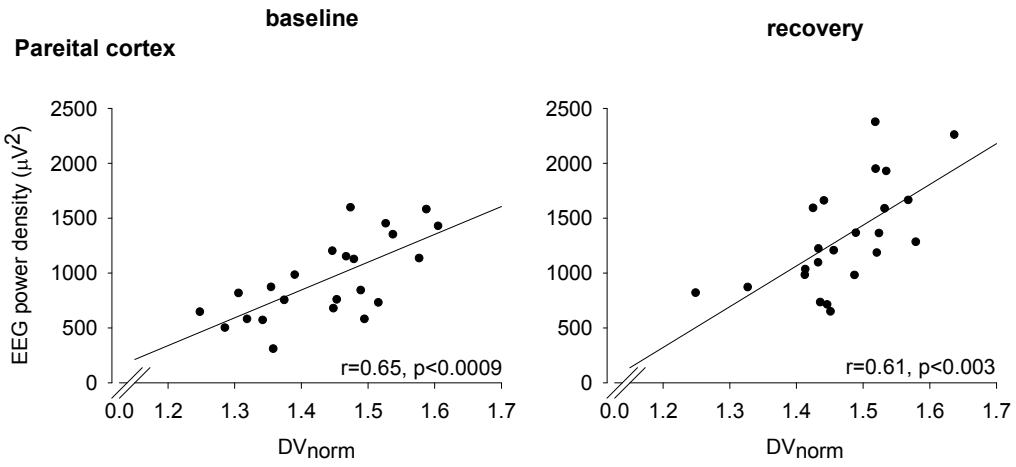

baseline
recovery
Medial temporal lobe
EEG power density (μV2)
DVnorm
r=0.68, p=0.0003
r=0.64, p=0.0009
Frontal superior medial
r=0.79, p<0.0001
r=0.68, p=0.0005
Orbitofrontal cortex
r=0.66, p=0.0006
r=0.66, p=0.0007
Dorsolateral prefrontal cortex
r=0.78, p<0.0001
r=0.75, p<0.0001


baseline
recovery
Parahipocampal gyros
EEG power density (μV2)
DVnorm
r=0.57, p=0.0045
r=0.34, p=0.1071
Insula
r=0.71, p=0.0002
r=0.55, p=0.0062
Amygdala
r=0.63, p=0.0013
r=0.51, p=0.0123
Thalamus
r=0.46, p=0.0263
r=0.33, p=0.1289



Regional specific correlations between mGluR5 availability and EEG slow oscillations (< 1Hz) in baseline (left) and recovery nights (right). The parietal cortex, precuneus, striatum, anterior cingulate cortex, medial temporal lobe, frontal superior medial, orbitofrontal cortex and the dorsolateral prefrontal cortex all survived 13 region Bonferroni corrections (< 0.0038) in baseline and recovery nights simultaneously.

Supplementary figure 2. Regional specific correlations between mGluR5 availability and EEG SWA.



Regional specific correlations between mGluR5 availability and EEG slow wave activity (SWA, 0.5 – 4.5 Hz) in baseline (left) and recovery nights (right). Only the parietal cortex survived 13 region Bonferroni corrections (< 0.0038) in baseline and recovery nights simultaneously.

Curriculum Vitae

Sebastian Camillo HOLST

Date of Birth	December 5, 1982
Place of Birth	Holbæk, Denmark
Nationality	Denmark

Education and Training Periods

11/2009- present	PhD student in Neuroscience (Human sleep research), University of Zurich PhD program of Integrative Molecular Medicine (imMed), Zurich
03/2010 - 10/2010	ESRS-EU "Marie Curie" Project (Project No. 046036) Training in Sleep Research and Sleep Medicine Bertinoro, Italy, 4-8 March, 2010 Seeon, Germany, 2-6 July 2010 Montpellier University Hospital Center, France, 11-16 October 2010
08/2003 – 05/2009	Master of Science in Engineering, Main field: Medicine and Technology, Technical University of Denmark and University of Copenhagen, Denmark
01/2007 - 01/2009	Member of the Academic Council (Akademisk råd), Technical University of Denmark
06/2007 - 08/2007	International Summer School Program, Ajou University, Suwon, South Korea
1999 - 2002	Technical High School (HTX), Holbæk, Denmark

List of publications

Katharina Hefti*, Sebastian C. Holst*, Judit Sovago, Valérie Bachmann, Alfred Buck, Simon M. Ametamey, Milan Scheidegger, Thomas Berthold, Baltazar Gomez-Mancilla, Erich Seifritz, and Hans-Peter Landolt (2012)
Increased availability of metabotropic glutamate receptor subtype 5 in human brain after one night without sleep.: Biological Psychiatry, 73(2): 161-168, 2013.
**Shared authorship*

Oral presentations at national and international meetings

Annual Meeting of the Swiss Society for Sleep Research, Sleep Medicine and Chronobiology, Aarau, May, 2013
"Sleep deprivation induced increases in mGLuR5 is linked to EEG markers of sleep need and dampened by genetically increased fragile X mental retardation protein"

(R)evolutions in Biology, Annual Meeting of the Life Science Society Switzerland (LS^2), Zürich, January, 2013
"Stimulant effects of caffeine: Pharmacogenetic aspects"

Pharmacology Poster day, Zürich, October 2012
"Metabotropic glutamate receptor subtype 5 (mGluR5) density and sleep- deprivation: A positron emission tomography study in healthy humans"

21st Congress of the European Sleep Research Society, Paris, France, September 2012
"Human polymorphism of the dopamine transporter modulates markers of sleep homeostasis and attenuates effects of caffeine following prolonged wakefulness"

Progress Report Seminar, Institute of Pharmacology and Toxicology, University of Zürich, May 2012
"FMR1, mGluR5 and the sleep EEG"

Progress Report Seminar, Institute of Pharmacology and Toxicology, University of Zürich, December 2011
"Dopaminergic neurotransmission and sleep homeostasis"

Annual Conference of the Swiss Society of Sleep Research, Sleep Medicine and Chronobiology & Swiss Neurological Society, St.Gallen, November 2011

"Effects of sleep deprivation and functional polymorphisms of DAT and COMT on EEG slow wave characteristics in NREM sleep"

ZIHP PhD-retreat, Jungfraujoch, June 2011
"Functional polymorphisms of DAT and COMT modulate slow wave sleep rebound after sleep deprivation in healthy humans"

Progress Report Seminar, Institute of Pharmacology and Toxicology, University of Zürich, December 2010
"Functional polymorphisms of DAT and COMT modulate slow wave sleep rebound after sleep deprivation in healthy humans"

20th Congress of the European Sleep Research Society, Lisbon, Portugal, September 2010
"Functional polymorphisms of DAT and COMT modulate slow wave sleep rebound after sleep deprivation in healthy humans"

Final symposium of ESRS-EU "Marie Curie" Project in Sleep Research and Sleep Medicine*, July 2010*
"Functional polymorphisms of DAT and COMT modulate slow wave sleep rebound after sleep deprivation in healthy humans"

Final symposium of ESRS-EU "Marie Curie" Project in Sleep Research and Sleep Medicine*, July 2010*
Chair of session "Oral session V. Basic research"

Poster presentations at national and international meetings

ZNZ Symposium, Zürich, September 2013
S. C. Holst, K. Hefti. J. Sovago, A. Buck, S.M. Ametamey, M. Scheidegger, R. Dürr, A. Baumer, B. Gomez-Mancilla, E. Seifritz, H.-P. Landolt
"Cerebral mGLuR5 availability and FMRP expression regulate homeostatic markers of sleep need in healthy men"

9th ZIHP Symposium, Zürich, August 2013
S. C. Holst, K. Hefti. J. Sovago, A. Buck, S.M. Ametamey, M. Scheidegger, R. Dürr, A. Baumer, B. Gomez-Mancilla, E. Seifritz, H.-P. Landolt
"Cerebral mGLuR5 availability and FMRP expression regulate homeostatic markers of sleep need in healthy men"

Pharma Poster Day, University of Zürich, August 2013

S. C. Holst, K. Hefti, J. Sovago, A. Buck, S.M. Ametamey, M. Scheidegger, R. Dürr, A. Baumer, B. Gomez-Mancilla, E. Seifritz, H.-P. Landolt
"Cerebral mGLuR5 availability and FMRP expression regulate homeostatic markers of sleep need in healthy men"

Swiss Society of Neuroscience Annual Meeting, Geneva, February 2013.
S. C. Holst, A. Bersagliere, V. Bachmann, P. Achermann, and H.-P. Landolt
"Sleep homeostasis and dopamine: Impact of genetic variation of *DAT* on the sleep EEG after sleep deprivation"

Pharmacology Poster day, Zürich, October 2012
S. C. Holst, A. Bersagliere, V. Bachmann, P. Achermann and H-P. Landolt
"Genetic variation of *Dopamine Transporter* contributes to individual effects of caffeine on the sleep EEG after sleep deprivation"

21st Congress of the European Sleep Research Society, Paris, France, September 2012
S. C. Holst, K. Hefti, J. Sovago, A. Buck, S. M. Ametamey, M. Scheidegger, R. Dürr, A. Baumer, B. Gomez-Mancilla, E. Seifritz, and H.-P. Landolt
Metabotropic glutamate receptor subtype 5 (mGluR5) availability and sleep deprivation: A positron emission tomography study in healthy humans

8th ZIHP Symposium, Zürich, August 2012
S. C. Holst, A. Bersagliere, V. Bachmann, P. Achermann, and H.-P. Landolt
"A variant of the dopamine transporter gene modulates markers of sleep homeostasis and the stimulant effects of caffeine in humans"

Annual Meeting of the Swiss Society of Sleep Research, Sleep Medicine and Chronobiology, Zürich, April 2012.
S.C. Holst, A.Bersagliere, V. Bachmann, P. Achermann, and H.-P. Landolt
"Genetic variation of DAT contributes to individual effects of caffeine on the sleep EEG after sleep deprivation"

ZNZ Symposium, Zürich, September 2011
S. C. Holst, A. Bersagliere, V. Bachmann, P. Achermann, and H.-P. Landolt
"Effects of sleep deprivation on EEG slow oscillations in NREM sleep: Modulation by functional polymorphisms of DAT and COMT"

7th ZIHP Symposium, Zürich, August 2011
S. C. Holst, A. Bersagliere, V. Bachmann, P. Achermann, and H.-P. Landolt
"EEG slow waves in NREM sleep: Effects of functional polymorphisms of DAT and COMT"